PHYSIOLOGICAL REVIEWS
VOL. 23 APRIL, 1943 No. 2

MALIGNANCY IN RELATION TO ORGANIZATION AND DIFFERENTIATION

N. J. BERRILL

Department of Zoology, McGill University, Montreal, Canada

Malignancy was tentatively formulated in a succinct report by the national cancer committee (Bayne-Jones, 1937) as a universal cell potentiality, varying in degree with cell type, of the nature of a somatic mutation, and resulting in automatic proliferation independent of a continuously acting provocative agent. It was asked "have they (the malignant cells) become 'fast' to the conditions which normally control cell growth in the body or is there a break in the internal control mechanism of the cell, or is there a loss in body control of cell activity? This, the core of the problem, has been almost entirely neglected."

The apparent neglect is clearly not lack of consideration. It is due to the standards of reference also being problems as challenging as malignancy itself. Malignancy and its associated phenomena merely constitute one aspect of growth, proliferation and differentiation of cells and cell constituents. The nature of the growth controlling forces of the body, of the internal control mechanism of the cell, the relation between nuclear change and cytoplasmic and organismic expression, the determination and maintenance of cellular specializations, and the mode of reproduction of proteins, mitochondria, enzymes, genes, and viruses are as little understood and equally challenging. The problem of malignancy becomes one of synthesis involving the problems of development as a whole. Clarification at any point is likely to throw light on the whole interrelated structure.

The outstanding feature of malignancy is that tumor growth is uncontrolled in relation to the host and shows little or no organization within itself. It is remarkably like that of normal tissues cultivated outside the body which grow in the same inchoate manner, tending to form marginal metastases and exhibiting a similar apparent decrease in degree of differentiation. In the sense used by Child (1915) malignancy becomes physiological isolation.

1. Growth controlling and organizing forces. The nature of the agency controlling and organizing the growth and multiplication of cells in an organism is obscure although its existence seems to be established (Sinnott, 1939). In the organism each cell develops in conformity with the character of its surroundings. Cues are decidely of supra-cellular origin (Weiss, 1940). The unlimited proliferation when cultured *in vitro*, the definite limits to growth of an organism under optimal nutritional conditions, the limits of growth and nature of regenerating tissues, and above all the growth rate curves characteristic of all organisms and of their constituent parts all indicate a subordination of cells within the organism to a control by the whole.

Various suggestions have been made concerning the nature of the controlling

agency but they suffer generally from vagueness and intangibility verging on the metaphysical. These, the field theories of embryology (Weiss, 1935; Waddington, 1935) have an undoubted descriptive value and are symbolic of a real quality of the organism, but the only attempts to translate the philosophic conception into chemical or physical terms, the metabolic gradient (Child, 1941) and the electro-dynamic theory (Burr and Northrop, 1935), have not met with ready acceptance. Yet it is startling to see the pattern of an organism change under the impact of a single substance, such as thyroxin upon amphibian metamorphosis. The field idea remains potent.

Field action may be considered as a tendency toward a certain equilibrium (Huxley, 1935). Even the mathematical rules governing the rate of regeneration bear a resemblance to those governing the restoration of electrical equilibrium between two regions at different potentials (Przibram, 1917). A limbbud grafted in abnormal position after the dorsoventrality is determined will undergo a gradual rotation toward a normal orientation, now that it is no longer sufficiently plastic to have the polarity of the whole imposed directly upon it (Harrison, 1921). Whether or not the controlling agency is a product of the cells it controls, cells or cell groups in an organized system acquire something distinctive of their position in the system.

It is commonly the case in regeneration from fragments for the regenerating tissue to proceed at a steadily decreasing rate until the original piece comes to occupy the same relative position in the new organism as it did in the old. In a significant number of cases there is a precision to a degree far beyond any biological need and unrelated to visible structural expression (Allen, 1921). Fragments of Hydra, placed more or less in contact, slide into positions so that each piece reoccupies approximately its original level in the organism (Papenfuss, 1934), while in everted Hydras the cells of the inner and outer layers slide between one another to regain their proper respective positions (Roudabush, 1933). Dissociated sponge tissues exhibit similar phenomena (Wilson, 1911; Brien, 1935, 1937). These examples are admittedly from lowly organisms but it is only in organisms of an order of size within range of cell dimensions that such phenomena can be expressed.

To what extent is this quality related to cell specialization? It is evident from the behavior of Hydra fragments and sponge dissociates that like cells and like tissues mutually adhere, and repulse unlike cells and tissues, while even homologous cells and tissues fail to cohere when taken from different species of sponge (Wilson, 1911) or Hydra. Specific and even racial differences in amphibians and in mammals may inhibit successful transplantation of tissues in mature animals, although embryonic tissues from different classes of vertebrates may be grafted together at least for a time. In amphibians a tissue incompatibility develops finally that significantly inhibits even autologous grafts (Harris, 1941).

It is significant that mammary tumors of mice exhibit similar compatibility phenomena, grafts within a given strain attaining almost 100 per cent success but very few to mice of another strain (Lewis and Lichtenstein, 1936). Barnes and Furth (1939) found that a mouse tumor originating in the reticulo-endo-

thelial system could be transplanted to every mouse of the ancestral leukemic stock but to no mouse of the ancestral non-leukemic stock, although it would grow steadily on chick-allantois. A somewhat similar situation exists for cellular constituents. While gonadotropic hormones of amphibia seem to be without specific effect, there undoubtedly exists a species specificity. The effectiveness of the hormones in a foreign species tends to vary directly with the phylogenetic proximity of donor and recipient, and the antibody nature of the sera is probably produced as an immune reaction to a foreign protein (Creaser and Gorham, 1939).

The protein basis and uniqueness underlying such phenomena probably relate in some way to the nature of the controlling agency. Yet during development the controlling system precedes cell and tissue specialization.

If malignancy is to be considered as a loss or weakening of the control of cell activity by such a system it is improbable that one cell should alone escape from the control. Multiple foci should be the rule rather than the exception. If malignancy is primarily due to a weakened control rather than an intrinsic change in the cells, transplantation of tumor cells to normal healthy hosts of the same species and race should fail to perpetuate the malignant growth. Such grafts should be incorporated or absorbed by the host system.

In frog embryos derived from overripe eggs tumor-like growths develop early, overrun and kill the embryo within a few days (Witschi, 1931). This and related phenomena led to an attractive speculation by Needham (1936) that overripeness of the egg leads to an upset of sterol metabolism, in turn leading to deviation of sex hormones upsetting the sex ratio, uncontrolled liberation of the embryonic evocator resulting in monster formation, and the production of chemically related carcinogens leading to epitheliomata.

More recent work confirms the observation that while multiple tumors do develop in the primary host embryos, they are due to a true weakening of the embryonic individuation field and a general reduction of proliferation rate. The tumors are the products of those cells that continue to divide at the normal rate, multiplying more rapidly than adjacent tissue and so assuming the form and effect of tumors (Briggs, 1940; Briggs and Berrill, 1941). When such a tumor is grafted into equivalent embryos of the same species it grows at the same pace as the surrounding tissue, loses its papillomatous character and is slowly absorbed by the host like many other grafts of normal tissue.

Weakness of extrinsic factors in the embryo therefore leads to a real malignant behavior by some parts, but there is no intrinsic malignant quality in the tumor tissue that can be expressed in a normal system.

2. Histogenesis and tumor extracts. There is considerable evidence that histodifferentiations generally develop according to location in the system. Position effects must operate so that a cell is directed along or made to select among its repertoire of potential specializations. The variety of special types varies with each kind of organism, but each kind remains distinct without grading into other types.

While the study of regenerates emphasizes the impact of position upon cell

destiny, an alternative mechanism is present in developing eggs, especially those of determinate or mosiac type.

It is generally considered that the inductors of vertebrate embryos are diffusible chemical substances. In the case of determinate eggs such substances arise precociously before significant diffusion becomes necessary, and are known as "organ-forming substances" (Conklin, 1905, 1929). Primarily they are specialized regions of the egg cytoplasm that are able to induce such rapid histogenesis that cleavage in these territories becomes inhibited and is limited to a few divisions (Conklin, 1905; Berrill, 1935). The inductors are liberated or activated at the time of rupture of the matured egg nucleus and resulting mixture of nuclear sap with the cytoplasm.

The inductor in amphibian embryos is considered by some to be a sterol (Waddington, Needham, 1936), by others to be protein (Barth and Graff, 1938), but more recently Holtfreter (1942) finds that all known agents act indirectly by causing cells to release the active substance the nature of which is completely obscure.

Sex hormones appear to act histogenetically and are known to be steroids. They are produced by gonadial tissue, even that of echinoderms (Donahue and Jennings, 1937) producing estrin as in the case of mammals and other vertebrates. Tissues in widely different parts of the body respond to these circulating secretions. According to Burns (1938) the sex inductors of amphibian larvae are identical with the sex hormones of the adult, and in these young forms they act through diffusion from cell to cell as do the inductors in the embryo. Each component of the developing ovotestis secretes its own hormone, to which it has a specific growth response. The hormone diffuses into the other component and the one that finally overrides determines the sex. Such substances are not specifically histogenetic since they arise in and affect tissues already more or less specialized. But it is significant that relatively specialized tissues secrete specific substances that promote the growth of the parent tissue. A similar case is the production of biotin by yeast, stimulating but not necessary to the growth of yeast.

The tissue response in these two instances is much like that of the circulating hormones. The immediate response in every case is an increase of proliferation in the tissue sensitive to the hormone. In most cases there is a specific differentiation. The specificities however appear to be associated with the responding tissue rather than the inducting substance. The inducting steroid is in the general circulation, reaching all tissues, but only certain related tissues are sensitive to it, while the histodifferentiation response is specific to each tissue responding. It is difficult to determine whether the specific differentiations are induced directly by the steroid or by the steroid-induced proliferation.

Hormones generally may be considered as tissue extracts, some originating in what appear to be residual tissues whose primary functions have been lost (Huxley, 1929), for example, thyroid from endostyle. Experimental support for this concept comes from Helff (1926) who found that perforation of the frog operculum enabling leg emergence is due to a hormone produced by underlying degenerating gills, and less intensely by tissues in general undergoing histolysis.

Zuckerman (1940) concludes that the idea of specific action of estrogenic hormones must be abandoned. He suggests that true Mullerian tissues respond by glandular proliferation and the epithelial metaplasia and stratification is anatomically a response of tissue in whose development estrogen-sensitive sinus epithelium has played a part.

The response is however even more general. Estrogen accelerates and intensifies the breakdown and ossification of growing cartilage, whereas the thyroid hormone increases proliferation and differentiation, with retarded ossification, of the same tissue (Silberberg, 1939). Histospecific responses appear therefore as the result of intensifications or retardations of each respective tissue cycle, as in the response of feather follicles to estrin and thyroxin (Lillie and Juhn, 1932).

Tissue cultures in vitro exhibit a loss of visible differentiation, related to the enhanced proliferation rate. This might be called dedifferentiation except that such tissues redifferentiate only their respective original character upon reimplantation (Strangeways, 1924). Mitotic multiplication inhibits the full expression of cell character, just as enforced differentiation inhibits cleavage (Berrill, 1935; Bloom, 1937).

Something persists through successive divisions in spite of the loss of structural and secretory indices, that maintains specific character. Either irreversible changes occur in the nuclei of specialized cells even though nuclear equivalence has been demonstrated in early developmental stages, or some substance is produced in the cell during the original differentiation that is not only an associate of its particular type of specialization but can act as an inductor of that special type. It may act directly on the cytoplasm or indirectly through nuclear activity.

Tissue maintenance factors and the so-called organ-forming substance of eggs may be identical and in effect be specific cytoplasmic selectors of nuclear repertoire, formed in the first place by nuclear reaction with relatively indifferent cytoplasm under the influence of a supracellular selector.

Malignant tissues frequently have been described as dedifferentiated or having reverted to an embryonic condition. In view of the real state in tissue cultures however it is clear that the high proliferation rate of tumor cells has a similar effect upon visible differentiation.

The question whether malignant cells have lost or gained something is relevant here, although it raises almost every aspect of cell function and constitution. Many instances are recorded of normal secretory functions persisting in malignant tissue. Mouse hepatoma secreted bile in primary and transplanted tumor (Strong and Smith, 1936). Fibroadenomata of rat breast secreted milk (Grauer and Robinson, 1932). Cartilage tumors produce a more or less normal ground substance (Purdy, 1938).

It is evident that malignancy in itself does not necessarily mask or destroy the basic cell character. If malignancy is the expression of something lost from the cell, the loss appears to be unrelated to the determinant of cell type.

The most striking examples of histogenetic tissue extracts come from malignant tissue itself. The reason for this is obvious. Malignancy implies unlimited growth. If to this is added a histogenetic effect, this effect will be ex-

hibited on a relatively enormous scale, although the malignancy has generally tended to overshadow this other quality. The transmission is exact, the same cell types, macroscopic features, etiological habits and degree of malignancy are induced by cell free extracts in the host as they appeared in the original tumor. The most obvious cases are those of tumors with the more striking histological character such as cartilage or bone. Cell free extracts of chondrosarcomas and osteochondrosarcomas of the fowl transmit their specific histology as well as malignancy (Rous, Murphy and Tytler, 1912; Muto, 1915; Furth and Breedis, 1937). Similar induction of malignancy together with specific histological character by cell-free tumor extracts has been demonstrated in mice (Parsons, 1936) and with less certainty in the frog (Lucke, 1938; Briggs, 1942), the former case from chemically induced primary tumors.

Malignancy in this light becomes one of the many properties of a cell induced in host cells by its noncellular extract. Similar extracts of nonmalignant cells might have similar induction properties, but without simultaneous conveyance of malignancy and consequent growth they remain indetectable.

Both components of a reacting system must be considered. Specific induction is to be correlated with a specific response. There appears to be a definite relative sensitivity of host tissues to extracts of related tissue types (cf. Barnes and Furth, 1939). Specialized cells are apparently unable to undergo true dedifferentiation or to acquire the power to redifferentiate along new line. The least specialized cells are likely to proliferate most since specialization and mitotic activity seem to be competitive exercises. Otherwise the induction-response system appears to be fairly definite. In chick embryos or membranes induction by sarcoma extract occurs only when in contact with mesodermal tissue. In adult fowl connective tissue cells react to sarcoma extract to reproduce the particular type of sarcoma extracted. The response is chiefly from sites of tissue derangement such as actively functioning ovary when injected intravenously or along the path of injury when into breast muscle. Muscle from susceptible fowl absorbs Rous tumor I agent, muscle tissue from nonsusceptible and nonmesenchymatous tissues from susceptible fowl do not (Claude and Murphy, 1933). Fowl endothelioma extract acts upon endothelial cells (Begg, 1927). According to Schiller (1937), a carcinomatous center infects adjacent epithelium but not connective tissue. The mammalian reticuloendothelial system reacts to extracts of lymphosarcomas (Parsons, 1938), while in frogs extracts of kidney adenocarcinoma appear to induce similar carcinomata in growing kidney tubules of other individuals (Lucke, 1938; Briggs, 1942). Lyophylized filtrates of mouse mammary tumor taken by mouth induce mammary tumors in Ax strains (Bittner, 1941).

In general there appears to be a relative sensitivity to tumor extracts of those less specialized tissues most closely related to the tumor tissue type. To this extent there is a close parallel with the endocrine-tissue reacting systems, and the problem may be one and the same.

Induction by tumor extract has been considered to be species specific, both for birds and frogs. If the parallel between the tumor extract reaction and sex

hormones reaction is significant, species specificity is to be associated with the carrier or protein component rather than the lipoid element. Tissue specificities develop during the course of development (Harris, 1941). Tissues can be blended in early stages that mutually reject one another at a later period.

Rous sarcoma extract normally fails to induce any significant response in ducks, but when massive injections are made into newly hatched ducklings malignant disease is invoked. Resistance becomes complete within a day or two of hatching. The question of susceptibility and resistance to tumor extract is probably as closely associated with protein specificities as is the degree of resistance to the grafting of normal and malignant tissues. Duran-Reynals (1942) reports more than an adaption of fowl sarcoma extract to ducks. In the chick the agent induces sarcomas and lymphosarcomas especially of liver and spleen but in the duck the same agent when successfully adapted produces widespread tumors of skin and digestive tract, and also growths in the skull, ribs and muscle, that is, primarily affecting epithelial and to a lesser degree mesenchymatous tissues. Adaptation to duck results in chick incompatibility, and the same procedure is necessary to produce reactions in the chick as is necessary to overcome duck resistance in the first place. The duck-modified agent now produces in the chick multiple bone tumors from periosteum and endosteum that invade the marrow cavity, also inducing nonmalignant new bone in the noninvaded parts.

The question arises whether the agent is actually a histogenetic inductor whose specific type is alterable, or whether it merely stimulates various tissues, according to their constitution, to proliferate and produce their respective growth types.

3. Teratomata. Teratoma is among the most complex and challenging phenomena of malignancy. It may be defined as tumors exhibiting little or no organization but containing many of the specialized tissue types. The phenomenon goes to the base of the problem of histogenesis and is clearly one of a malignant tumor exhibiting in part epigenetic development. The problem is threefold, how do the specialized tissues arise, what is the significance of the apparent absence of organization, and what is the significance of the malignant character of the growth. An adequate answer to all of these would raise embryology to the status of a classical science.

Barron (1916) described pineal teratoma as consisting of muscle fibres, goblet cells, cartilage, bone, glandular, squamous and columnar epithelia. Similar tumors are reported arising in thyroid (Putsch and Nelson, 1935) and suprarenal tissue (Geschichter, 1935), and more generally from gonadial tissue of either sex. The limited mixed types usually containing cartilage described for the palm (Simard, 1937) breast (Binkley and Stewart, 1940) and liver (Bullock and Curtis, 1925) are probably aspects of the same phenomenon.

Teratoma testis has been produced experimentally by a number of workers (Bagg, 1936; Falin, 1940) through the combined action on the testis of zinc salts and normal or injected sex hormones. According to Champy and Lavedin(1939) seminomas and embryomas may be induced by almost complete removal of the

testis. A typical tumor consists of a stroma of embryonic mesenchyme which forms hyaline cartilage, often replaced by bone of endochondral origin, and muscle, glandular ducts lined with goblet cells or columnar epithelium, islands of keratinized stratified epithelium, feather follicles with feather germs, and medullated and nonmedullated neurones, with obvious areas of carcinoma and adenocarcinoma. Needham (1936) compares it to the chaotic condition when an individuation field is thrown out of existence, when a "field" at some point fails to control evocator (inductor) substances. Falin (1940) considers that at the site of zinc injection necrohormones, trephones, etc., are liberated during cellular disintegration that act on pluripotential testicular cells like the inductors or evocators of embryos, and that lagging or misplaced gonocytes are responsible for all teratomas. He criticizes Michalowsky's (1932) suggestion that teratoma testis arises from spermatogonia and that the spermatogenic cells of the tubules approach the ovum in capacity. He finds it necessary to assume parthenogenesis of spermatogonia and finds it difficult to account for teratomas elsewhere.

According to Holtfreter's recent work (unpublished) the embryonic inductor responsible for neurulation is liberated from damaged cells, as imagined by Falin, but this inductor is not considered by embryologists to initiate development as such, nor is parthenogenesis conceived as applying to any cells other than fully grown ova in the throes of polar body formation. Experimental or natural parthenogenesis has never been observed in öogonia or partly grown öocytes.

Two kinds of cells have the capacity for complete or partial development, fully mature ova and entirely unspecialized totipotent cells. These types are poles apart and their confusion is responsible for much loose terminology used in the interpretation of teratoma.

Spermatozoa and mature ova are each in their own way extremely highly specialized types of cell. It is part of the special nature of the fully formed egg that when properly stimulated it undergoes rapid segmentation as a consequence of its large size, and simultaneously undergoes a series of specialized form changes associated with regionally developed special cytoplasmic differentiations (cp. Needham, 1933; Berrill, 1935). Together these produce the embryo with its peculiar characteristic early stages. Immature ova lack both properties and have no capacity as such for development. To speak of parthenogenetic development of either öogonia or of spermatogonia is in terms of embryology nonsense.

There is presumably a phase, however, in the production of öogonia and spermatogonia when the cells are entirely unspecialized, before even their respective destinies to produce oöcytes and spermatocytes have been determined. Such unspecialized types of cells may exist in many parts of the body. In lower vertebrates and most invertebrates unspecialized tissue consisting of multipotent or even totipotent cells occur throughout the organism, and are responsible for the regenerative capacities of these forms. Except for the progressive specialization of tissues in higher vertebrates, there is no *a priori* reason why such unspecialized types should not persist locally and in small numbers in these as well.

Under certain circumstances such cells or cell groups are capable not only of regenerative development, as in amphibians, but of total development as in tunicates. The course and conditions of development however are not the same as those of developing eggs. Physical or physiological isolation from the parental organism is essential for total development in the one case, similar isolation of the part from the whole is necessary in the other. Then if proliferating cells are able to adhere together and are continously supplied with adequate nourishment, development of organized differentiating structure proceeds. It is necessary to emphasize that in the absence of a relatively large quantity of material to start with, as is present in even the smallest egg, these cells divide because they have grown beyond the normal limit and are permitted to do so by their immediate cellular environment. The invocation of inductors or evocators is neither more nor less serviceable than it is for all malignancy. They may be necessary to account for certain kinds of histogenesis even in teratoma, but only of histogenesis of cells already present or multiplying. They do not account for growth itself.

In virtually every account of teratomas the fact of malignancy is more or less taken for granted, the assumption being that parthenogenesis of mature ova in the ovary, or the miscalled parthenogenesis of öogonia and spermatogonia, account for the type of growth. Distorted development may be explained by the abnormality of the site of development, but teratology is not malignancy. Either malignancy develops in one or more tissues as development proceeds, which is conceivable, or malignancy exists from the beginning and development is its outcome.

There is little doubt that on occasion mature ova develop parthenogenetically in the ovary, for the most part forming benign teratological growths. Under abnormal developmental conditions spontaneous malignant changes might well take place in one or more tissues. The problem would be that of spontaneous tumors generally.

In the case of teratomas appearing at other sites parthenogenetic development, even if it had meaning here beyond mere proliferation, would fail to account for the growth. Totipotent or multipotent cell groups do not grow or develop unless isolated in a real though not necessarily physical sense from the rest of the organism. Otherwise they do not grow except as an orderly constituent of that organism, and when grafted elsewhere become resorbed. Growth of non-ovarian teratomas must accordingly be malignant growth from the very beginning. The problem therefore is that of the malignant change itself and of the peculiar teratomal development that follows. To a considerable extent it is a matter of emphasis.

If a malignant change occurs in a relatively unspecialized cell one of several ways may be taken. Slight specialization invisibly expressed may result in spindle cell sarcomas, as from fibroblasts, and in the case of tumors of the testis seminomas arise probably from cells already too specialized to be multipotent. Cells entirely unspecialized may become actual rather than potential spermatogonia by virtue of their location. If such cells become malignant they may conceivably merely proliferate without other change of character, appearing as

seminoma types or even as so-called chorionepithelioma. On the other hand it is clear that malignancy so affects any cell that it behaves as if isolated from the organism. If multipotency or totipotency is innate in such a cell, and if all nutritive requirements for unlimited growth are met, as they are, the conditions for the expression of any independent developmental capacity are fulfilled.

It is important to emphasize once more the dissociability of fundamental developmental phenomena, histogenesis, cell multiplication and growth, and morphogenesis (Needham, 1933). Histogenesis may be considered by itself. One of the major mysteries of development is the mechanism by which an indifferent cell gives rise to a progeny of diversely differentiated types. More is known of the induction of neurones from such cells and all that is certain here is that the inductor can be liberated from damaged or necrotic cells by an unrelated variety of agents. But the products of necrosis do call forth neurogenesis.

In a growing mass of indifferent cells, among which many must be degenerating and dying, necrotic by-products must be plentiful and healthy cells of indifferent type may be induced to undergo initial segregations into a few simple types. These may in turn interact or follow through their own respective destinies of further segregation. In any case the histogenetic situation is comparable to that of embryos and the problem of tissue segregation one and the same. Obviously the malignant quality does not inhibit the formation or existence of the various cell types.

At the same time it is impossible to ignore the existence of certain rudiments of organization, and while organization itself requires explanation, even rudimentary organization may account for histogenetic segregation without calling on necrotic hormones.

In advanced teratoma testis of the fowl feather follicles and glandular ducts are definitely organized structures, and it is evident that certain types of cells are able to cohere and undergo unitary development associated with their respective type. An experimental analysis of the general relationship between histodifferentiation and malignancy in teratomas is greatly needed. All that can be said is that the most specialized tissues proliferate slowest and are therefore the least malignant. But the non-transplantability of teratomas needs further investigation. It is possible that in the course of tissue segregation certain types are able to inhibit whatever underlies the malignant quality, and when proliferation is almost or entirely suppressed all the latent capacity for organization and differentiation within that mass of cells is expressed, the nature of the expression being determined by the degree of segregation previously attained.

The other evidence of organization is found in the earliest stages of teratomal growth and in its essentials is amazingly similar in renal (Masson, 1938) testis and some ovarian tumors (Peyron, 1939). Masson has given clear account of the development of nephrogenic tumors, while Peyron's descriptions, as distinct from his interpretations, demonstrate the essential similarity of testis teratoma development. According to Masson blastematous cords of the kidney gives rise to minute vesicles which he considers to be the sole and earliest tissue of origin

of the nephrogenic tumors. Each vesicle buds off other vesicles or cell masses which give rise progressively to a group of teratomatous tissues, including neurons, striated muscle and nephrons. He concludes boldly that the origin of the blastematous cords from the neuroepithelium of the embryo accounts for all the cell types encountered except muscle and nephron and that therefore in the embryo certain muscle and nephrogenic tissue must come from the mesectoderm of the neural crest and not from the mesentoderm, thus reversing the trend of interpretation from embryology to tumors! It is implied that consequent on malignancy certain cells of neural crest origin progressively segregate into their prospective histological types. Their fate had already been somewhat determined so that multipotency persists, rather than the totipotency of prospective spermatogonia.

The formation of vesicles that in turn bud off other vesicles and cell masses is common to both testis and kidney teratomas and must represent organization of a kind. Peyron considers those of testis to be blastocysts budding as in polyembryony and giving rise to chorioplacental masses, embryomas, and much malignant chorioma, stages and tissues plausibly named only if an origin from true parthenogenetic ova is valid. This cannot be established.

In those forms, e.g., ascidians, where a comparison can be made, there is a profound difference between the development of an egg and that of a group of totipotent indifferent cells (Berrill, 1941). The egg cleaves to form a blastula, gastrula, tadpole larva, etc., before transforming finally into a miniature adult. The somatic cell group in contrast multiplies and forms a small vesicle which by extensions, foldings and subsequent histodifferentiation develops directly into a similar miniature adult without passing through any of the so-called historical phases of development.

Blastocysts, embryonic discs, fetal membranes belong to "historical" aspects of egg development, however essential they may actually be for normal development. There is no more reason to expect development from unspecialized cells in a mammal or bird to yield the early stages of egg development than in the lower chordates. It is more reasonable to compare the primary vesicles of teratomas with the primary vesicle of asexual ascidian development. Totipotent cells of higher vertebrate if able to develop at all might be expected to exhibit features comparable to the primary phases of bud development than to those of any developing egg.

The repeated vesicular budding and final disintegrations may well be the result of malignancy. The following description of teratoma may accordingly be made.

The malignant quality acquired by one or a group of indifferent totipotent cells of probable epithelial pedigree acts as an isolating factor in relation to the rest of the organism, enabling it to exhibit whatever developmental potency it may possess. Proliferation begins, uncontroiled by the parent organism as in the case of malignant growth generally. Morphogenesis begins with the formation of a small vesicle. Either by virtue of its tissue environment or more likely because of the innate new quality of its component cells the system breaks down, first by segregating daughter vesicles and cell masses and then by complete dis-

integration. The various cell strains initiated by this effort express the primary malignancy in various degrees, the least structurally specialized cells retaining the highest multiplication rate and therefore becoming the most malignant, such as epithelioma.

The original malignant agent induces development and at the same time is responsible for its subsequent breakdown.

Tumor induction. One of the most confusing aspects of malignancy is the diversity of the agents capable of inducing it, radium, x-ray, ultra-violet, polycyclic hydrocarbons, sex hormones, tumor extracts, butter yellow, arsenic and viruses. We are faced with two alternatives. Either each kind of agent produces its own peculiar change in the cell and a large variety of qualitatively different changes enable cells to behave as though isolated from the parent organism, or, directly or indirectly, every such agent induces finally the same basic change in a cell, that is, a variety of stimuli call forth one and the same reaction. It is relevant to recall that many chemically and physically unrelated agents similarly evoke one and the same organizer response in amphibian embryos, all acting probably through the liberation of necrotic by-products.

Both views are plausible. A variety of agents may in various ways induce the same qualitative change in a cell, uncontrolled growth being the result of the change in quality, or a variety of qualitative alterations produced by the various agents may call forth the same reaction if they exceed a certain magnitude.

The malignant response and the proliferative response of normal cells to a disturbance of the condition of equilibrium as in regeneration and tissue culture must be considered together. Malignancy cannot be understood until much more is known concerning both cell division and its inhibition in tissues. Actual division of a cell is the climax of a complex process of growth and duplication of a multitude of cell components, while the inhibition of the process in vivo is clearly not due to lack of nutritive substances or to any deficiency on the part of the cell.

Such evidence as there is suggests that normal cells refrain from or are prohibited from continuing division by surface conditions, by their relationship to one another. In a tissue they are under the control of the external agency to which they themselves probably contribute. Normal proliferation ceases when a condition of equilibrium is reached.

There may be much more than an analogy between the induction of malignant behavior by chemical agents in tissue cells and the induction of divisions in eggs by parthenogenetic chemicals.

Eggs admittedly are relatively giant cells and anything that restores to them the metabolism of normal tissue cells may be expected to start a series of divisions tending to restore typical somatic cell dimensions (Whittaker, 1933; Berrill, 1935). But apart from this there are the significant facts that the most effective parthenogenetic agents are surface active compounds such as oxybutyric acid, that profound physical or physico-chemical changes occur immediately in the egg cortex, and that division asters are apparently induced *de novo* within the endoplasm.

Since there appears to be a lower size limit to any given type of cell, all such cells must necessarily keep growing in order to continue to divide, whether under the impact of the regenerative stimulus or of a carcinogen. The question arises whether the cells are induced to continue growing and consequently keep dividing to maintain optimum dimensions or whether they are induced to divide and must grow to maintain the same surface-volume ratios.

Tumor cells are generally described as being somewhat larger than their normal counterparts with large nucleoli (Lewis, 1938). It may be significant that similar features characterize tissues preparing for or in process of regeneration (Sayles, 1931). Nuclei become vesiculate, nucleoli large and multiple, and cell size markedly increased, almost as though an overdrive is established.

Lipoids, mitochondria, sex hormones, steroid carcinogens, avian tumor agents, and parthenogenetic chemicals are all surface active compounds and may be expected to affect primarily cortical or surface conditions of a cell. Any permanent abnormality in cortical regions may well so affect the intercellular relationships of a cell that it may be impossible to attain a state of equilibrium. Cortical cytoplasm and cortical relationships may be affected directly by external agents or indirectly by factors operating through their effect on nuclear constitution.

Of the various tumor inducing agents, radium and x-ray almost certainly primarily affect the nucleus. According to the dosage these produce either cell and nuclear destruction, permanent inhibition of mitotic division, or chromosome mutation. Induction of malignancy in somatic tissues occurs with much the same order of incidence and dosage as in the case of germ cell mutation. There is in this fact considerable support for Boveri's theory, which so far has neither been proven nor disproven.

Obvious chromosomal irregularity is probably incompatible with survival beyond a few cell generations, but slight irregularity compatible with continuing bipolar mitotic divisions is at least conceivable and yet such cells may be so changed that malignant behavior results. The more recent concept of somatic gene mutation does not differ basically from Boveri's chromosome theory. All that is required in either case is that some intra- or inter-chromosomal change occurs that permits continuation of normal division processes and at the same time so changes a cell that it behaves as a physiologically isolated unit. It may be entirely a matter of chance whether any alteration of this type induced by x-ray, radium or ultra-violet leaves the division mechanism unimpaired and is of a kind expressing itself as malignancy.

Colchicine apparently affects nuclear division in a non-differential manner, retarding or temporarily inhibiting the process during a critical phase. Since x-rays seem absorbed and produce their dislocation effects during the more condensed chromosome phases, a combined use of colchicine and x-ray should produce a much higher tumor incidence than the same x-ray dosage alone. The negative result reported by Brues, Marble and Jackson (1940) does not appear to be conclusive.

The chromosome aberration hypothesis, whether in the form of chromosome

dislocations or of gene mutations, raises the general question of the dependence of cell character on the persisting integrity of the whole nuclear constitution. Much depends upon the interpretation of the amitosis phenomena. Until amitosis is demonstrable in living cells it is difficult to accept its existence as fully established. At the same time a pattern tends to emerge from the incidence of its reported occurrences. In all cases where it is established as a probability the cell types appear to be structurally relatively simple but highly specialized in chemical function. It is typical of proliferation in decidua and embryonic envelopes of vertebrates, the periblast of meroblastic ova, follicle cells in the ovary, endosperm, etc., of flowering plants (Belar, 1928). It is described as the typical method of multiplication of bone cells (Bast, 1921), urodele erythrocytes (Charipper and Dawson, 1928), frog erythrocytes (Ferrari, 1931) and of mature mammalian cartilage (Elliot, 1936). It is suggested by Child (1907) and Elliot that it replaces mitosis when the demand for certain requisite metabolites such as oxygen exceeds the supply.

Unless amitotic division masks unsuspected examples of endomixis and intranuclear bipolar segregation, it must result in qualitatively unequal division, and since typical cell character is maintained it follows that only a partial representation of full nuclear constitution is necessary for the continued amitotic division and special functioning of certain kinds of tissue. This is in contrast to the need for normal nuclear constitution for development and histogenesis and raises the whole question of the origin of cell differentiations and specializations and their maintenance. Obviously greater effort should be made to establish or disestablish amitosis as a fact, or to determine its true nature. If it is what it seems to be, and if chromosome aberration can lead to malignancy, tumors should frequently arise in the above mentioned tissues. They do not as a rule, though Butterworth (1937) suspects x-ray induced granulosa-cell tumors of mice to arise from nests of follicle cells after degeneration of ova.

On the other hand there is considerable indirect support for the chromosome aberration hypothesis, which in any case is by no means an exclusive theory.

According to Ishibasi and Harada (1933) the chromosome numbers in a series of human and animal tumors are in accord with the idea of chromosome disharmony being related to tumor development; though the question always recurs whether the relationship is a causal one or whether tumor formation and chromosome aberration are both products of some deeper disturbance.

Carcinogenic cysticercus cysts induce irregular division and chromosome abnormalities almost immediately upon application and long before any other indication of malignancy can be detected (Mendelsohn, 1935). Mitoses were found to be bipolar in spite of a much greater variability of chromosome number than in normal tissues.

According to Huskins and Hearne (1936), the relatively high chiasmata frequency of the gonads of cancer-susceptible mice strains indicates a greater susceptibility in these strains to mitotic disturbance generally, that the processes of chromosome "splitting", spindle formation and cytokinesis are near the borderline of lack of correlation. From the study of maize, Jones (1940) con-

cludes that chromosome reallocations, genes removed from some cells and replicated in others result in permanent alterations involving growth, color, size, shape and composition, that it is "now evident why external agents such as physical radiations, carcinogenic chemicals, hormones, viruses, parasitic organisms and what next, can produce the same result as inherited tendencies, either by increasing the frequency of chromosome breaks and relocations or by altering the growth-regulating regions in the chromosomes."

Associated with chromosome aberrations is the widespread occurrence of multipolar mitoses in tumors. As they are not invariably present in tumor tissue their presence has been considered by many to be a result of abnormal conditions and degeneration within the tumors, and that cells exhibiting such mitoses are not the malignant cells *per se* and have but a short life ahead of them. This of course could be true without necessarily excluding multipolar mitosis as a causal factor.

There are two distinct problems: does the phenomenon precede malignancy or only follow it, and how is it to be interpreted in itself. Only in the case of experimentally induced tumors is there much likehood of determining whether multipolar mitosis precedes malignancy. The evidence must inevitably remain ambiguous in the case of spontaneous tumors.

The general effect of steroid carcinogens on growth of the whole animal appears to be retardative (Haddow and Robinson, 1939) and the immediate effect on certain tissues at least is the induction of frequent multipolar mitoses. Since there is on an average a latent period of several months between application and first detectable signs of tumor, it is virtually impossible to consider such mitoses the product of malignancy. Similarly a high incidence of multipolar mitoses was found to be a more or less immediate response to the presence of the Crocker cysticercus cysts, although here again there is a long latent period between cyst infection and tumor origin (Mendelsohn, 1934).

There appears to be little doubt that in two outstanding types of tumor inductors the tumors arise from tissues in which cell proliferation has continued for a relatively long period with varying degrees of abnormality. Boveri's hypothesis is definitely supported by the facts and it is impossible to dismiss these abnormal mitotic phenomena as mere by-products of established malignancy.

It does not follow that tumors arise from the progeny of cells that have undergone multipolar mitosis, although it is possible. Multipolar mitosis may be an extreme case and other cells may react in other and less obvious ways. Thus according to Hearne Creech, (1939) in fibroblast cultures exposed to carcinogenic hydrocarbons chromosomes showed a precocious splitting in prophase, recalling the correlation between chiasmata frequency and mammary tumor susceptibility (Huskins and Hearne, 1936).

In general carcinogenic hydrocarbons are growth inhibitors, non-carcinogenic hydrocarbons are not (Haddow and Robinson, 1939). Similarly there is a growth retardation and degeneration, and lack of stimulation, when mouse and rat fibroblast cultures are exposed to methylcholanthrene (Earle and Voegtlin, 1938). Accordingly there is a probability that multipolar mitoses are more

likely to arise through the suppression of a cytoplasmic cleavage than from a precocious doubling of the centrosome; that they arise through differential retardation.

Boveri's suggestion of lagging chromosomes with consequent deficiencies or reduplications in daughter cells lies somewhere between the multipolar condition and chiasmata formation. In the case of multipolar mitosis chromosome distribution to daughter cells is likely to be too abnormal for prolonged survival, and the two milder types of aberrant chromosome behavior are more likely to be compatible with the survival of proliferating cells. Bipolar mitosis is probably a requisite. The range of variability of chromosome number in tumors (Ishibashi and Harada, 1933) suggests lagging chromosomes and is hard to correlate with the types of segregation that would probably follow the multipolar condition.

The fact that multipolar mitoses are so commonly a feature of malignant tissues has naturally led to the suggestion that maligancy is basically a disturbance of the centrosomal mechanism (Lewis, 1938). In our present state of abysmal ignorance concerning the nature of the centrosomal force it is impossible to accept or to dismiss this hypothesis. Fogg and Warren (1940) found shortly after exposure of Walker rat tumor to x-ray that centrioles per cell increased to 4, 6 and 8 in up to 60 per cent of the cells, suggesting the predominance of one or more incomplete divisions, binucleate cells and multipolar mitoses being frequent. If there is a real disturbance of the centrosomal mechanism, it apparently relates to their activity in cytokinesis rather than their rate of reproduction.

At the same time there may be a significant parallel between the induction of multipolar mitoses by carcinogenic hydrocarbons and the action of surface-active chemicals (cp. Tyler, 1941) as inductors of accessory centrosomes in the eggs of various marine invertebrates.

Orderly progressive division of nuclear constituents however is possible without spindle, plate, or breakdown of nuclear membrane, i.e., endomixis (Painter and Reindrop, 1939) and may be followed by simultaneous division restoring the diploid condition (Berger, 1938). There may also be an equivalent division of the centrosomes (Pollister, 1939).

As in the case of the vertebrate "organizer", the attractive hypothesis of chemical inductors of specific structural type has slowly yielded to a non-specific conception. The cyclopentane ring and C20 methyl group typical of steroids and bile acids are shown to be unconnected with their carcinogenicity (Fieser, 1936). According to Morton, Branch and Clapp (1936) one and only one structural similarity is evident, all have at least four benzene rings. Either four rings, or a single ring modified by its neighbors, must be present, for benzene as such has never been shown to produce cancer. Structure apparently cannot be the common factor relating the carcinogenicity of the various types of hydrocarbon, and maybe there is no common denominator. Fieser (1938) while more attracted to the idea of a definite chemical reaction states that there is no *a priori* reason for supposing that hydrocarbon carcinogenicity is not entirely

physical. Studies on the interaction of polycyclic hydrocarbons and steroids in surface films (Clowes, Davis and Krahl, 1939) indicate that interfacial forces may be strong enough to permit the hydrocarbons, when present in animal tissues, to influence the physiological function of cholesterol and other sterols

In view of this, the fact that tumor cells have generally a higher lipoid content than most other types (Yasuda and Bloom, 1932) becomes highly significant, especially in view of the importance of lipoids as components of the surface layers and structural features of cells as well as in cell metabolism. Lipoids are a major constituent of mitochondria and, according to Graaffi (1939), carcinogenic hydrocarbons have a selective affinity for these bodies.

Accordingly these hydrocarbons conceivably may act as malignant agents in two ways. By so altering, qualitatively or quantitatively, the general steroid metabolism of the cell the processes of mitotic division may be differentially affected resulting in aberrant chromosome distribution in some degree, or by altering in some manner the steroid complex so that surface relationships are permanently changed.

It may be relevant that steroids themselves, as estrogen, can induce malignancy in estrogen-sensitive tissue. It is possible, perhaps probable, that the induction is indirect, that overstimulation of the tissues results in an occasional chromosome mutation or reduplication of a type producing malignant behavior. There is however evidence to the contrary. According to Gardiner (1936) male mice receiving small amounts of estrogen possess mammary glands resembling those of normal virgin females and develop very few mammary tumors, while those receiving larger doses show abnormal growth from the beginning and develop many mammary tumors. There is an implication that malignancy is the climax of growth abnormality. Loeb and his co-workers (1936) emphasize this view in connection with malignancy of the reproductive tract. In vagina and cervix, with increasing age, there is a lateral extension of surface epithelium leading to folds, presumably under the influence of estrogens. Injection of estrogen intensifies and accelerates this process. The growth is not localized but is wide and of multiple areas. Step by step it passes to a precancerous (sic) condition and to beginning cancer. Very similar phenomena are reported for guinea-pig peritoneum following estrogen injections (Lipschütz, 1942). We have then this view, widely held by pathologists, that malignancy does not arise from a simple cell focus but is usually preceded by definite though vaguely described changes over a relatively wide area of tissue, and final malignancy may arise in multiple foci. This is supported by the effect of injections of cell-free extract of lymphosarcoma in mice (Parsons, 1938) which produced mass changes in the lymphatic system.

Self-synthesizing proteins of an alien type, as in the case of the infective papillomata virus of the wild rabbit, may stimulate growth locally. The majority of virus infections, while exhibiting a significant organ specificity, have no such effect and it may be that the particular protein type associated with rabbit papillomata affects surface relationships directly or indirectly whereas others do not. The heavy protein in this case stimulates infected epidermal

cells to abnormal growth and extracts of these induce similar growths in domestic rabbits (Kidd, 1938). Extracts of these in turn are innocuous, and while inducing antibodies do not possess heavy protein in detectable quantity. It seems likely that in the domestic form the virus protein introduced in the extract is able to induce a proliferative response but is itself unable to reproduce in the domestic rabbit tissue. The pathogenicity of the wild type extract is destroyed by ultra-violet but not its ability to bind complement, indicating interference with its reproductive capacity. The occasional invasive malignancy of the papillomata may be properly compared with the induction of malignancy by estrogen. In both cases malignancy may be either the culmination of a progressive abnormality of growth, or it may be a distinct and more or less spontaneous malignant mutation in a favorably proliferating tissue.

Nature of malignancy factor. Whatever the inductive agent may be it is probable that the final change in the cell directly responsible for malignant behavior is of a fairly specific kind. Cell proliferation is itself primarily a response to changing surface relationships and to place the onus of continuing division directly on a derangement of components of the mitotic mechanism such as the centrosome or to accuse genes regulating the normality of mitosis of misbehavior is to ignore the significance of size and growth.

Cell multiplication implies protoplasmic growth except in the case of most eggs, enormous cells, with abnormal surface conditions, capable of subdivision into smaller and smaller cells until cortical and metabolic conditions are returned to normal. Tumor cells are no smaller and at the most are but little larger than their normal counterparts. Cell division must therefore occur when the cell size exceeds a certain critical value, else tumor cells should get progressively smaller and disappear. A cell divides primarily to retain certain volume-surface ratios of the whole and of its parts, and average cell size can be regarded as a form of equilibrium between essential surface activities and the mass of material. This applies not only to the cell as a whole but to all levels of its protoplasmic constitution. An alteration of surface conditions could affect the equilibrium so that it is reached at a different value and a larger or smaller average cell size might be established. According to Dodge (1937) methylcholanthrene has such an effect upon yeast cells.

The factor for malignancy must accordingly satisfy the following requirements. It must either, directly or indirectly, so affect cellular interrelationships that cells are constantly endeavoring to attain a condition of tissue equilibrium that cannot be reached in the normal tissue environment with their new cortical constitution, or certain cellular components, if not all, continue to grow indefinitely and continued cell division is an inevitable response to increasing cell size. And it must be intimately associated, if not identical, with the agent determining the type of cell differentiation.

Claude (1940) in an analysis of high speed centrifuge extracts of both avian tumor tissue and normal embryo finds particulate components of an average size of 70 millimicrons and of the general constitution of a phospholipid-ribonucleoprotein, therefore unlikely to be nuclear fragments. In the case of the chicken

sarcoma amounts of this sedimented material as small as 10^{-9} grams induce typical sarcomas upon injection. It has been stated that chicken tumors induced by carcinogenic hydrocarbons could not be transmitted by cell-free extracts (Mellanby, 1938), although non-infective extracts injected into rabbits produce sera which neutralized filtrates of Rous sarcoma I (Fould, 1937). Experiments on a somewhat limited scale however show that there is no fundamental difference between the so-called chicken virus tumors and chemically induced tumors (Amies, Carr and Purdy, 1939). Dibenzanthracene added to hanging drop cultures of chick embryo fibroblasts and then infinitely diluted produced a culture that produced tumors upon injection, the particulate deposit from cell free extracts transmitting the tumor type readily. Similarly mouse sarcomas induced by dibenzanthracene and grafted for many generations have yielded cell-free filtrates capable of transmitting the respective tumor types in an impressive number of cases (Parsons, 1936, 1938).

Claude's suggestion that mitochondria are involved in the malignant change is plausible. They are closely associated with structural differentiation of cells, are actively related to surface structure and properties, are intimately concerned with many aspects of cell metabolism, and are independent self-perpetuating units. What little evidence there is suggests that they are concerned in the initial determination of cell character (Conklin, 1905, 1929). They are disposed in a cell so as to supply a maximum surface, so that the mitochondrial-cytoplasmic interface must be a significant architectural feature.

The general rôle of cell division and multiplication relative to malignancy may have been overemphasized. In a limited sense it may be, and probably is, basically important since the malignant cell appears to be radically and permanently different from its normal counterpart, and changes of all kinds are more apt to occur during the delicate and intricate procedure of division. Yet all the various peculiarities of cell multiplication and differentiation associated with malignancy may well be the consequences and not the cause of continuing protoplasmic growth. In a developing organism growing tissues may be undergoing rapid cell multiplication, slow multiplication or no cell division at all, and yet the rate of growth is the same whatever the degree of cellulation and follows the same curve to end in a "steady state" applying to the whole (Berrill, 1941). The fundamental problem is the nature of the agency creating such an end point and the course of growth leading to it. It is not a matter of nutrition. Malignant tissue grows no faster than normal tissue can when explanted (Schrek, 1936; Hoffmann, Tenebaum and Doljanski, 1939), and it is the failure to reach stability that is the crux of the problem. Whether continuing growth, autocatalytic or otherwise, of a single component such as mitochondria, involves growth of protoplasm as a whole is not known. Tumor metabolism is significantly different from that of normal tissues. According to Druckrey (1935) and Fleischmann (1936), it is the metabolism of injured tissues, while Dean Burk (1937) defines a tumor as a growing glycolyzing tissue with a deficient respiration. The problem seems to be infinitely complex and appears to be the nature of protein synthesis and reproduction, its relationships to cell surface

qualities and general metabolism, and the susceptibility of the whole to the inhibitory control of organization, whatever the basis of this may be.

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PATHWAYS OF GLYCOLYSIS

ALBERT DORFMAN

The Chemical Laboratory of the Walter G. Zoller Memorial Dental Clinic, University of Chicago

The last decade has witnessed tremendous progress in the understanding of the processes of carbohydrate metabolism. The combined efforts of many laboratories have resulted in the formulation of a fairly complete picture of the pathway of glycolysis in muscle extract and fermentation in yeast extract. Additional studies on many types of cells have suggested that there exists a common pathway widely distributed in nature for the formation of pyruvic acid from polysaccharides and hexoses. The pyruvic acid thus formed may be reduced to lactic acid, decarboxylated, or oxidized by a great variety of reactions which are only partially understood at the present time.

The frequent occurrence of these reactions, leading to the formation of pyruvic acid, in nature raises the question as to whether this scheme represents the sole pathway for the formation of the key intermediate pyruvic acid. From time to time various workers have suggested that other pathways, particularly of a non-phosphorylating nature, are responsible for glycolysis in various tissues, especially tumor, brain and embryo.

It is with these questions that this review will principally concern itself. Part one will consider the evidence for alternate pathways of glycolysis in animal tissues, and the question of the presence or absence of phosphorylation in such tissues. Relevant observations on the metabolism of other types of cells will be touched upon in part two.

As a framework for the discussion, the Meyerhof-Embden-Parnas-Cori-Warburg cycle is presented in figure 1. This series of reactions, for purposes of brevity, will be referred to solely as the Meyerhof cycle throughout this review. The reactions in figure 1 will not be discussed in detail since several recent reviews have covered this material (15, 16, 50).

Students of cellular metabolism are constantly faced with the fundamental difficulty of attempting to achieve understanding of *in vivo* reactions by the use of *in vitro* methods. Brei and tissue slice techniques have frequently been used to simulate physiological conditions. Such techniques have been employed to a very large extent in studies on alternate pathways of glycolysis. The use of breis and tissue slices, although possibly being more physiological than the use of extracts, introduces other complications. Thus such preparations do not allow free diffusion of added substrates, making quantitative or qualitative changes in the suspending medium difficult to interpret. In addition, the simultaneous occurrence of large numbers of reactions makes elucidation of pathways almost impossible. The alternative procedure has been to use tissue extracts and to attempt to study individual reactions by separating the components of such extracts. Such studies, when accompanied by adequate rate studies and tissue analyses, can at best give information as to which reactions can occur in intact tissue. The best understanding is obtained only by a comparison of the results

of both methods, especially with respect to rates, intermediate compounds, and effects of inhibitors.

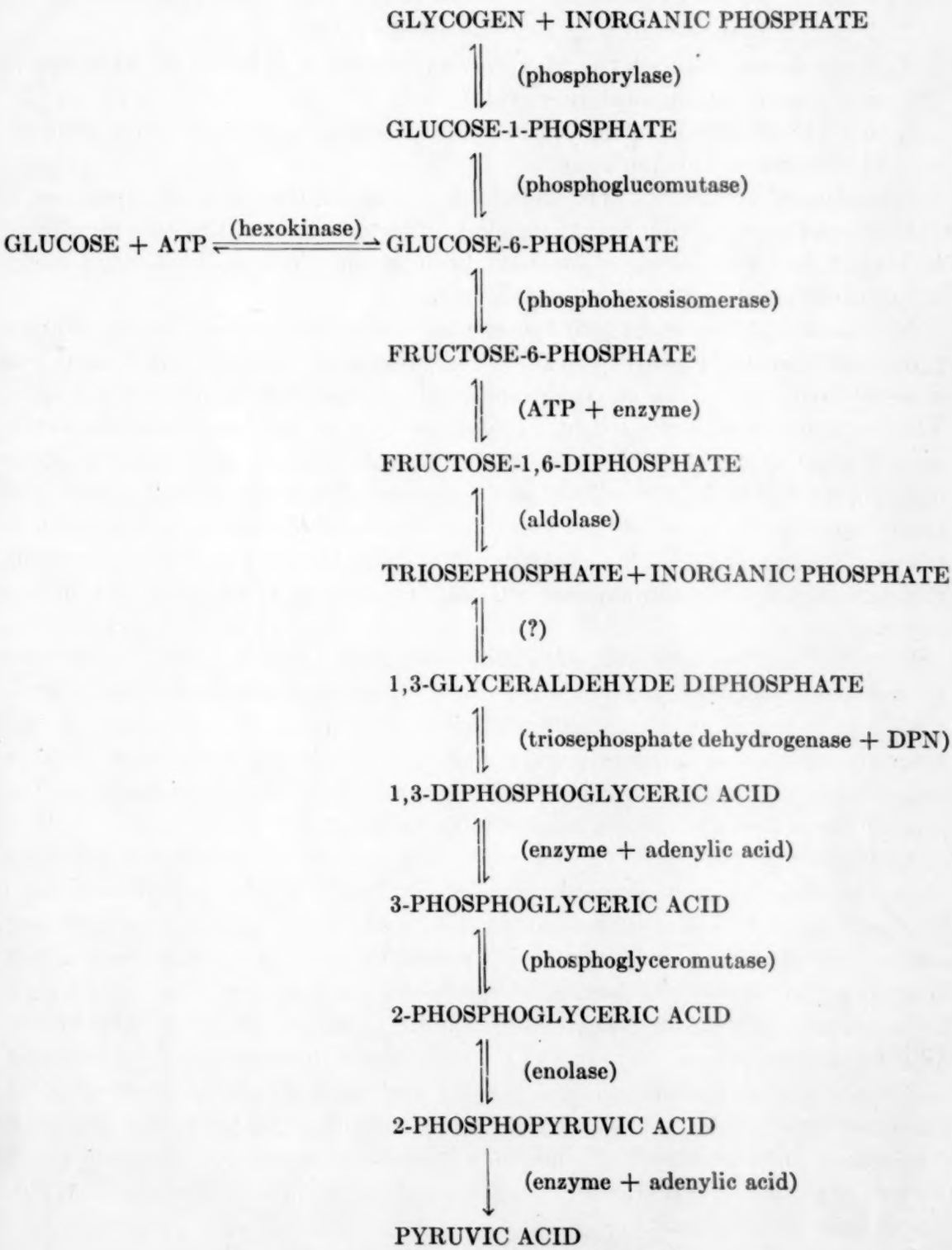

Fig. 1. Meyerhof-Embden-Parnas-Cori-Warburg cycle

Since certain types of experiments have been repeatedly performed to prove or disprove the occurrence of alternate pathways of metabolism, it seems wise to

analyze the literature in terms of types of evidence, rather than types of tissues. Each group of experiments will be analyzed according to the following criteria:

1. Does the evidence prove the existence of the Meyerhof cycle?
2. If not, is it consistent with the Meyerhof cycle?
3. If not consistent with the Meyerhof cycle, does it disprove the existence of any type of phosphorylating cycle?
4. If a non-phosphorylating pathway is indicated, does the evidence indicate the nature of this pathway?

Selectivity of substrates. The difference in the relative rates of glycolysis of various substrates by different tissues has attracted the attention of a number of investigators. Such observations have been partially responsible for the postulation of different pathways of metabolism.

Needham and Nowiński (56) found that intact five day old chick embryos produced lactic acid rapidly from glucose and mannose but were usually unable to produce lactic acid from glucose-6-phosphate, hexosediphosphate, or glycogen. This work has been criticized by Macfarlane (40) on the basis that the results were due to the fact that the phosphorylated sugars and glycogen were unable to diffuse into the cells. Needham and Lehmann (58) have partially answered these objections by showing that the added hexosediphosphate is broken down to triosephosphate, and that this change occurs within the cells since the suspending medium does not contain aldolase although extracts of embryo are rich in this enzyme.

Meyerhof and Perdigon (51, 52) have recently been able to prepare extracts of both rat and chick embryos which are able to bring about the production of lactic acid from hexosediphosphate plus pyruvic acid in the presence of fluoride, the proper coenzymes, and a phosphorus acceptor. This finding leaves no doubt that embryo tissue contains the essential triosephosphate dehydrogenase for the production of lactic acid from hexosediphosphate.

Needham *et al.* (57), however, have been able to prepare breis which will bring about the dismutation of hexosediphosphate and pyruvic acid only when adenyl triphosphate (ATP), and diphosphopyridine nucleotide (DPN), are added, but will actively glycolyze glucose without these additions. It would seem as if this difference may be due to a lack of adequate phosphate acceptors in Needham's preparations. Thus when hexosediphosphate is glycolyzed, the addition of ATP results in its hydrolysis by ATP phosphatase to adenylic acid which can then act as a phosphate acceptor and permit the necessary dephosphorylation of phosphopyruvic acid. When glucose is being used, no added phosphate acceptor is necessary since glucose itself acts as a phosphate acceptor. It would be of interest to determine whether any phosphate acceptor could replace ATP in the experiments of Needham.

The failure of intact embryo to glycolyze glycogen is hardly significant since this observation may be due either to lack of penetration into the cells, or absence of the necessary phosphorylase for the breakdown of glycogen.

The lack of rapid utilization of the phosphorylated hexoses and of glycogen by brain presents a situation analogous to that in embryo. Thus the earlier

work by Ashford and Holmes (2) was done with brain brei, and is open to the criticism of permeability. Recently it has been possible to prepare extracts of brain (22, 23, 64), which can utilize the phosphorylated hexoses as well as glucose. Geiger (22, 23) showed that concentrated extracts of brain contain an inhibitor which prevents glycolysis, but on dilution such extracts readily convert glucose to lactic acid. Geiger's work indicated that small amounts of hexosediphosphate increase the rate of formation of lactic acid from glucose, and that phosphate is esterified during the course of glucose utilization. Peculiarly enough, he found that no lactic acid formation occurred from the intermediate phosphorylated compounds of the Meyerhof cycle, although phosphate was required for lactic acid formation from glucose. Ochoa (64), however, was able to prepare extracts from brain which utilized the phosphorylated compounds and glucose at about the same rate. The difference between the results of Ochoa and of Geiger may be due to the lack of phosphate acceptors in Geiger's preparations, since Ochoa showed that addition of phosphate acceptors increased the rate of glycolysis of hexosediphosphate as well as the rate of dismutation between hexosediphosphate and pyruvic acid.

Tzusuki (79), Matsuzaki (42), and Harrison and Mellanby (28) have all claimed that tumor tissue is unable to utilize phosphorylated hexoses at a rate comparable to glycolysis of glucose. In contrast, Boyland and Boyland (12) have been able to show rapid glycolysis of phosphorylated intermediates by tumor extract.

In summary it must be concluded from this group of experiments that tumor, brain, and embryo tissue contain the requisite enzymes for utilizing the phosphorylated hexoses which are intermediates of the Meyerhof cycle. These experiments then indicate that the Meyerhof cycle can occur in these tissues and furnish no evidence to indicate that an alternate non-phosphorylating pathway is instead operative.

Summation. Summation experiments have frequently been employed in determining whether two substrates are metabolized via the same pathway. If two substrates are glycolyzed by the same enzyme system, then the total lactic acid production in the presence of optimal amounts of both substrates should not exceed that obtained from the compound used most rapidly. However, if there is a difference in glycolytic pathways, the lactic acid production in the presence of both substrates should be the sum of that obtained in the presence of each substrate separately. It should be noted, however, that a difference in just one step in glycolysis of the two substrates is all that is necessary to produce evidence of summation, providing that step is the slowest in the chain of reactions. Thus, summation between glucose and glycogen does not indicate alternate pathways of glycolysis if one assumes the conversion of glycogen to glucose-6-phosphate (via Cori ester) or the phosphorylation of glucose to glucose-6-phosphate is the slowest step in glycolysis in any particular tissue.

Ashford (3), using rabbit brain brei, found summation of lactic acid production from glucose and glucose monophosphate and from glucose and hexosediphosphate, while he found no summation from glucose and mannose. On the basis

of these experiments he concluded that glucose and mannose are glycolyzed by the same set of enzymes while glucose and the phosphorylated hexoses are not converted to lactic acid through the same pathway. In these experiments glucose was used more rapidly than hexosediphosphate. The fact that the addition of hexosediphosphate, to a preparation glycolyzing glucose at a maximum rate, increases the lactic acid production, can readily be explained on the assumption that under these conditions the phosphorylation of glucose is the slowest of the series of reactions necessary for lactic acid production. The increase in rate of lactic acid production from hexosediphosphate, above that obtained from glucose alone, by the addition of glucose cannot be explained on the same basis. However, the glucose may speed up the formation of lactic acid from hexosediphosphate by acting as a phosphate acceptor. As has been previously stated, Ochoa (64) and Meyerhof (51) have shown that the rate of dismutation of hexosediphosphate and pyruvic acid is increased by the addition of suitable phosphate acceptors necessary for the dephosphorylation of phosphopyruvic acid. Summation of lactic acid formation from glucose and hexosemonophosphate can be explained on the same basis if one assumes that the conversion of hexosemonophosphate to the diphosphate is a relatively slow reaction compared to the initial phosphorylation of glucose. An indication of the possible validity of such an explanation is that Ashford (3) obtained partial summation with hexosemonophosphate and hexosediphosphate. Thus it is possible that the results of Ashford can be accounted for within the framework of the Meyerhof cycle.

Geiger (23) found no summation of lactic acid production from glucose and glycogen by diluted brain extract.

B. E. Holmes (30) observed summation with glucose and hexosediphosphate using tumor brei, while Boyland, Boyland, and Greville (11) found no evidence of summation with glucose and hexosediphosphate using a tumor extract. The results of Holmes are similar to those of Ashford, the possible explanation of which has already been discussed. It is of interest to note that Boyland *et al.* added adenylic acid, which is able to act as a phosphate acceptor. Holmes points out that the addition of ATP increases the rate of lactic acid production from hexosediphosphate but not from glucose. These observations are consistent with the interpretation that the glycolysis of hexosediphosphate requires the presence of a phosphate acceptor.

Bumm and Fehrenbach (9, 10) claimed that a difference in glycolytic pathway exists between skeletal muscle and diaphragm. These workers, using a brei of skeletal muscle, observed no summation with glycogen and glucose plus hexokinase. However, with rat diaphragm glycolyzing the same substrates, summation was observed. They concluded that diaphragm, in contrast to skeletal muscle (red), has two different pathways for dealing with glucose and glycogen. The results of these workers can be explained within the framework of the Meyerhof cycle if one assumes that the initial phosphorylations are the slowest steps in the process. The difference obtained between diaphragm and skeletal muscle may reflect a greater concentration of phosphorylase in the skeletal muscle.

Although experiments on summation using brei do not provide positive evi-

dence for the exclusive operation of the Meyerhof cycle, they do not negate this possibility, and furnish no evidence for non-phosphorylating glycolysis. Experiments on extracts of brain and tumor support the interpretation that glycolysis of glucose proceeds through the same pathways as does glycolysis of the phosphorylated hexoses.

Glyceraldehyde. Much of the evidence in support of non-phosphorylating glycolysis rests on conclusions drawn from the use of glyceraldehyde as an inhibitor. Mendel (46) showed that *dl*-glyceraldehyde at a concentration of 10^{-3} *M* inhibits almost completely lactic acid formation by tumor, but has little effect on respiration of tumor or normal tissue (liver, kidney, and brain). Mendel, Bauch and Strelitz (47) found that the inhibition by 10^{-3} *M* glyceraldehyde is completely reversed by 10^{-3} *M* pyruvate and partially reversed by as little as 2.5×10^{-5} *M* pyruvate. Ashford (4) found that *dl*-glyceraldehyde also inhibited glucolysis. In the same year E. G. Holmes (32) confirmed the work of Ashford, and also showed that the production of lactic acid by muscle from either starch or glucose plus hexokinase is not inhibited by this compound. Needham and Lehmann (60) found that *dl*-glyceraldehyde inhibited glucolysis and mannolysis of chick embryo about 90 per cent, and that this inhibition was reversed by pyruvic acid.

On the basis of these results Needham (35), Ashford (4), and Tzusuki (79) have concluded that *dl*-glyceraldehyde is a specific inhibitor for non-phosphorylating glycolysis. This view has been contested by Adler and co-workers (1), Boyland and Boyland (12), Macfarlane (40), Süllmann (77), and more recently by Stickland (74).

The interpretation of much of the data on glyceraldehyde inhibitions is difficult. Thus Boyland and Boyland (12), Süllmann (76), and Adler (1) claim that *dl*-glyceraldehyde inhibits the glycolysis of glycogen, starch and glucose plus hexokinase by various tissues, while Needham and Lehmann (35, 60), B. E. Holmes (30) and Baker (7) found no such inhibitions. The following factors are possibly responsible for these discrepancies: 1. Different concentrations of glyceraldehyde have been used by different workers. 2. Lehmann and Needham (35) have shown that when *dl*-glyceraldehyde is dissolved a dimeric form exists in solution. This dimer inhibits the conversion of glycogen to Cori ester. On standing, however, a dissociation to the monomeric form apparently occurs, and the solution no longer inhibits the phosphorolysis of glycogen but inhibits glucolysis by chick embryo. This finding introduces a difficulty in assessing papers published before 1938 when this factor was recognized. 3. Stickland (74) has recently shown that the degree of inhibition of muscle extract, glycolyzing glucose with the aid of yeast hexokinase, is dependent upon the concentration of hexokinase. He also found that the critical concentration of hexokinase varies with different extracts.

Considering the factors listed above, certain general conclusions can be reached. Glucolysis of brain, tumor and embryo preparations is inhibited by low concentrations of *dl*-glyceraldehyde (below 0.01*M*). The inhibition of glucolysis by muscle extract in the presence of yeast hexokinase is dependent on the concentra-

tion of hexokinase. Inhibitions of glycogen and starch glycolysis require much larger concentrations of *dl*-glyceraldehyde and are probably due to the dimeric form.

If lactic acid formation in embryo, brain and tumor tissues proceeds along the same pathway as does glycolysis in muscle, what underlies this marked difference in sensitivity toward glyceraldehyde? Meyerhof *et al.* (49) showed that the aldolase of muscle extract will bring about the irreversible condensation of *d* or *l*-glyceraldehyde with dihydroxyacetonephosphate to form *l*-fructose or *l*-sorbose phosphates respectively. These esters do not give rise to the equilibrium mixtures of hexose-6-phosphates. Macfarlane (40) suggests that in muscle and yeast the amount of dihydroxyacetonephosphate available for combination with glyceraldehyde is large, while in tumor, brain and embryo it is small. Thus, in muscle, the glyceraldehyde is readily removed and is unable to bring about inhibition, irrespective of mechanism of the action of glyceraldehyde. This view has been contested by Süllmann (77) and Stickland (74). The former found that the rate of removal of glyceraldehyde from muscle extract glycolyzing glycogen is insufficient to account for lack of inhibition. Stickland pointed out that if Macfarlane's explanation were correct, then the more rapidly glycolysis proceeds, the greater should be the glyceraldehyde disappearance, with ultimate lifting of the inhibition. The opposite was found to be the case, i.e., in muscle glucolysis proceeded at a much more rapid rate than starch glycolysis; nevertheless, the inhibition of glucolysis was much greater than the inhibition of lactic acid production from starch. A second objection raised by Stickland is that if the glyceraldehyde were being removed as glycolysis proceeds in the muscle extract, the inhibition should decrease with time, while in fact he found an increase with time.

The work of Stickland (74), Süllmann (76, 77) and Adler (1) suggests that *dl*-glyceraldehyde inhibits the phosphorylation of glucose. Their observations also indicate an alternative explanation for the variation in tissue susceptibility to glyceraldehyde; for inhibition of glycolysis by glyceraldehyde is most striking in those tissues in which glucose is the principal substrate for glycolysis. This suggestion is attractive in that it provides a rather simple explanation for the observed differences between the two groups of tissues. The findings of Mendel (46) and Baker (7) present a possible objection to this interpretation. Both workers found that concentrations of glyceraldehyde which inhibited anaerobic glycolysis by tumor tissue did not affect the rate of oxidation of glucose by these tissues. It has generally been assumed that the oxidation of glucose by animal tissues is dependent upon preliminary phosphorylation. If glyceraldehyde inhibits this initial phosphorylation, inhibition of respiration should parallel inhibition of glycolysis. Three possible explanations of this discrepancy suggest themselves. The first is that aerobic phosphorylation of glucose may occur through a different mechanism than does anaerobic phosphorylation. It is interesting to note that Loebel (38) and Dickens and Greville (17, 18) found that fructose could be rapidly oxidized by brain brei, but is not glycolyzed anaerobically. Gerard and Meyerhof (25) made a similar observation on nerve. A second

possibility is that glyceraldehyde inhibits the phosphorylation of glucose-6-phosphate to hexosediphosphate rather than the initial phosphorylation of glucose. The latter suggestion is made less likely by the fact that Stickland (74) obtained reversal by a hexokinase preparation, which presumably esterifies glucose to glucose-6-phosphate. Another objection to this interpretation is that Süllmann (77) found no inhibition of lactic acid formation from glucose monophosphate. A third possibility is that under aerobic conditions a reaction occurs which results in the removal of *dl*-glyceraldehyde.

Any explanation of the mechanism of glyceraldehyde inhibition must explain reversal by pyruvic acid. Mendel *et al.* (47), using tumor brei, found that pyruvic acid reversed glyceraldehyde inhibition of glucolysis. Holmes (32) observed no pyruvic acid reversal with brain slices. Baker (7) confirmed the results of Mendel with tumor tissue, observing partial reversal of glyceraldehyde inhibition when the glyceraldehyde inhibition was complete, and more complete reversal at lower glyceraldehyde concentrations. She found, as did Holmes, no reversal of the inhibition of brain glycolysis. Needham *et al.* (60) noted that the glyceraldehyde inhibition of embryo glycolysis could be partially reversed by pyruvic acid.

The mechanism by which pyruvic acid reverses glyceraldehyde inhibition has received no adequate explanation. It is known that glyceraldehyde in large concentrations reacts with pyruvic acid (through the mediation of the appropriate enzyme and coenzyme), in the same manner as does glyceraldehydephosphate. This reaction can remove glyceraldehyde, irrespective of the mechanism of glyceraldehyde inhibition. Süllmann (77) found that the inhibition of glycogen glycolysis by muscle extract by 0.1 *M* glyceraldehyde could be reversed by 0.022 *M* pyruvate, with lactic acid production. This is consistent with the above explanation. However, Stickland (74) was unable to find any evidence of reaction between glyceraldehyde and pyruvic acid, when both reactants were present in a concentration of 0.003 *M*.

Another possible explanation for the reversal by pyruvic acid depends on the interpretation that glyceraldehyde inhibits metabolism by inhibiting phosphorylation. It is possible that the phosphorylation in tumor and embryo is linked with the reoxidation of reduced diphosphopyridine nucleotide. In such a case the presence of pyruvic acid might serve to reoxidize the reduced diphosphopyridine nucleotide and directly or indirectly to overcome the inhibition. It is of interest to note that Baker (7) found that aerobic glycolysis is more resistant to glyceraldehyde inhibition than is anaerobic glycolysis. The lack of reversal in brain, observed by both Baker and E. G. Holmes, may be due to a difference in mechanism of phosphorylation in brain.

By the use of partially resolved glyceraldehyde, Needham and Lehmann (60) showed that the *l*-glyceraldehyde is responsible for inhibition of embryo glycolysis. This observation has been confirmed by Mendel, Strelitz and Mundell (48) working with tumor tissue. Süllmann (77), working with lens extracts, found the *d*- and *l*-forms equally active. He worked, however, with much larger concentrations of glyceraldehyde.

Needham and Lehmann (60) also showed that the *d*-form is changed non-enzymatically to methylglyoxal, which is converted to lactic acid by methylglyoxalase. This observation is of interest in view of the fact that most workers have reported that glyceraldehyde inhibition is not complete even at high concentrations. Such results are probably due to the formation of lactic acid from the glyceraldehyde. Baker (7) observed a stimulation of glycolysis in liver and kidney which may also be due to lactic acid production from *d*-glyceraldehyde. Stickland (74) noted a similar increased CO_2 liberation from liver in the presence of glyceraldehyde, but found that only a small part of this was actually due to lactic acid, determined chemically. He attributed the manometric results to the formation of glyceric acid by the aldehyde mutase in liver.

The literature of glyceraldehyde inhibition has been reviewed in considerable detail since many workers have regarded such evidence as of paramount importance in proving that glycolysis in certain tissues does not proceed through the pathway demonstrated for muscle extract. At this time it is not possible to conclude that the work on glyceraldehyde inhibition provides positive evidence for the existence of the Meyerhof cycle in the tissues under discussion, but such experiments do not necessitate the postulation of alternate pathways. It is obvious that, aside from certain minor discrepancies, the inhibition of glucolysis by glyceraldehyde is consistent with lactic acid production through phosphorylated intermediates. In no case is it possible to conclude that the work on glyceraldehyde provides positive evidence for the existence of a non-phosphorylating pathway.

Fluoride. Sodium fluoride has been frequently used in studies on the mechanism of glycolysis and fermentation. The earlier literature on fluoride inhibition has been reviewed by Gemmill (24). The mechanism of fluoride inhibition has recently been elucidated by the studies of Warburg and Christian (81). These workers have separated enolase (80), the enzyme responsible for the conversion of 2-phosphoglyceric acid to 2-phosphopyruvic acid, and found that its active form exists either as the zinc, manganese or magnesium salt. The zinc enolase is inhibited by hydrocyanic acid, while the magnesium enolase is very sensitive to fluoride. The zinc and manganese salts are also inhibited by fluoride, but at considerably higher concentrations. The degree of inhibition of the magnesium enzyme was found to be dependent on the relative concentrations of magnesium, phosphate, and fluoride.

Differences in sensitivity to fluoride have been used by some workers as evidence to prove the existence of either non-phosphorylating glycolysis, or at least the existence of alternate pathways of glycolysis. Thus Ashford and Holmes (2) found that formation of lactic acid from glycogen and from glucose by brain brei differ in sensitivity to fluoride. They also found that fluoride did not inhibit the appearance of free phosphate to the extent expected when glucose was used as substrate. A possible explanation of these effects is that fluoride indirectly inhibits the phosphorylation of glucose by brain as a consequence of its effect on enolase. Thus, if the enolase reaction is stopped, phosphopyruvic acid is not formed and ATP cannot be resynthesized, resulting in the inhibition of

glucose esterification. This interpretation is supported by the work of Ostern, Baranowski and Terszakoweć (65), who found that sodium fluoride inhibited the formation of adenosine triphosphate in yeast extract from adenosine and phosphoglyceric acid.

Scharles, Baker and Salter (72) found that 0.05 *M* fluoride did not affect lactic acid formation from hexosemonophosphate by tumor extracts. Since much lower concentrations of fluoride (0.001–0.005 *M*) generally inhibit glycolysis by muscle extract these workers consider this observation as indicative of a different pathway of metabolism. In view of the finding of Warburg and Christian that the sensitivity of enolase is dependent on the magnesium and phosphate concentration, it is difficult to rule out the validity of the Meyerhof cycle in tumor tissue on the basis of differences in fluoride sensitivity.

Scharles *et al.* (72) also found that their tumor extracts were able to convert hexosephosphates to lactic acid, with no sensitivity to fluoride at 52°. It is likely, however, that in this case the results were due to non-enzymic conversion of triosephosphate to methylglyoxal which was in turn converted to lactic acid by methylglyoxalase. It is doubtful whether such a pathway is of any physiological significance.

Kerly and Bourne (34), using retinal extracts, found that smaller concentrations of fluoride are required for the inhibition of glucolysis than are required for lactic acid formation from hexosediphosphate. The explanation of the results of Ashford and Holmes would seem to apply equally well to those of Kerly and Bourne.

Needham *et al.* (57) failed to observe the expected accumulation of phosphate esters during the glucolysis of embryos poisoned with fluoride, although an increase in the hexosediphosphate fraction is evident in some of their data. Somewhat similar observations were made by B. E. Holmes (31), who noted that 0.001 *M* fluoride inhibited the formation of lactic acid from glucose, but that no esterification of phosphate occurred. These results again are consistent with the Meyerhof cycle if one considers the possibility of indirect inhibition of phosphorylation by fluoride.

Needham *et al.* (57) have claimed that with a chick embryo preparation inhibition of glucolysis did not occur until a concentration of fluoride of *M*/50 was reached, while with the same preparation *M*/200 fluoride completely inhibited the enolase reaction (phosphoglyceric→phosphopyruvic). Macfarlane (40) has suggested that this finding may be due to accessibility of the enolase at the cut surface to both the phosphoglycerate and the fluoride. However, when glucose is used as substrate, the cells must be penetrated by fluoride for inhibition. The validity of such an interpretation lacks experimental support. The observation of Needham is unusual in that other workers have usually found glucolysis to be more sensitive to fluoride than the glycolysis of phosphorylated intermediates (Ashford and Holmes (2), B. E. Holmes (31), Kerly and Bourne (34)).

Werkman and co-workers (83, 86) have shown that *Propioniibacterium pentosaceum* can be grown on a medium containing large concentrations of sodium fluoride, and retain the ability to ferment glucose while becoming insensitive to

fluoride. Such organisms are unable to ferment phosphoglyceric acid. The parent strain is able to ferment both glucose and phosphoglyceric acid and both fermentations are inhibited by fluoride. This work suggests that the "trained organisms" are able to ferment glucose through mechanisms not involving phosphoglyceric acid. Similar results have been reported by Stone *et al.* (75) for *Aerobacter aerogenes.*

On the whole, the work on fluoride gives little reason to doubt the existence of the Meyerhof cycle in mammalian tissues. The observations of Needham, which cannot be obviously explained within the framework of the accepted cycle lack confirmation. The observations in any case do not prove the existence of a non-phosphorylating pathway.

The work of Werkman and co-workers indicates that, in micro-organisms, alternate pathways do exist, but again, there is no evidence in support of a non-phosphorylating pathway. No such claim has been made by the authors.

Ashford and Holmes (2), studying the effect on glycolysis of removing free phosphate by adding an excess of calcium chloride to rabbit brain brei, found that such treatment inhibited the glycolysis of glycogen but not that of glucose. It is hardly likely that complete removal of phosphate could be achieved by such treatment, but a decrease in phosphate concentration did occur. Addition of phosphate restored acid production from the glycogen. A possible explanation of these results without contradiction of the Meyerhof cycle depends upon the fact that the phosphorylation of glycogen is dependent upon the concentration of inorganic phosphate (14), while the phosphorylation of glucose is accomplished with phosphate derived from ATP. Thus a decrease in the concentration of inorganic phosphate would affect glycolysis of glycogen to a much more marked extent than that of glucose.

Needham (59), performing similar experiments on embryo brei, found that excess calcium chloride had no effect on glucolysis. The formation of lactic acid from glycogen was not studied. No analytical evidence for the complete removal of phosphate is given. Macfarlane (40) and Macfarlane and Weil-Malherbe (41) have recently pointed out the extremely small quantities of phosphate that are required for glycolysis in both intact yeast and brain slices. The phosphate is continually regenerated and thus acts in catalytic quantities. They have also shown the extreme difficulty involved in the complete removal of phosphate. The literature to date contains no conclusive demonstration of mammalian glycolysis in the absence of phosphate.

Several workers have attempted to prove the absence of the Meyerhof cycle by studies of the ability of the various tissues to bring about the successive steps of the cycle. Many older studies are of little value since they do not consider recent modifications of the cycle.

The most detailed study along these lines has been that of Needham and Lehmann (58) on chick embryo. They found that embryo possessed all of the necessary enzymes with the exception of the triosephosphate dehydrogenase. Similar claims have been made by Tsuzuki (79) concerning tumor tissue. However, Meyerhof and Perdigon (51, 52) have recently been able to demonstrate the

presence of this enzyme in adequate amounts in extracts of both chick and rat embryos. Similarly the presence of the necessary enzymes has been demonstrated in brain (64), tumor (11, 12) and retina (34).

From these observations it would seem that, whether or not the principal pathway of glycolysis in these tissues is through the known phosphorylated intermediates, the necessary enzymes for this pathway can be extracted from these tissues. Thus the Meyerhof cycle cannot be ruled out in the tissues under discussion on the basis of lack of the necessary enzymes.

The possibility of oxidation of carbohydrate without previous phosphorylation is suggested by the work of Harrison (27) and of Hawthorne and Harrison (29) on the glucose dehydrogenase of liver. This author has been able to extract an enzyme from beef liver which brings about the oxidation of glucose to gluconic acid. It apparently is able to function with either di- or triphosphopyridine nucleotide as coenzyme. From the work of Harrison and of Rice and Dorfman (67), this enzyme does not appear to be identical with the hexosemonophosphate dehydrogenase of Warburg. The importance of this enzyme in carbohydrate metabolism remains to be determined.

Much has been written of the effect of the structural organization of the cell upon the pathway of metabolic reactions, but as yet little experimental evidence is available along these lines. This subject has been discussed in a recent review by Commoner (13). Of particular interest is the observation of Gaddie and Stewart (20) on intact heart muscle. These workers found that, on repeated contraction, the carbohydrate stores could be depleted. The addition of certain substrates restored contraction. Among these was found methylglyoxal. This observation is of interest in that methylglyoxal can be both produced (21) and used by muscle (20, 39) and suggests that this compound may be capable of furnishing energy for contraction.

The question as to whether the Meyerhof cycle is operative in normal muscular contraction has been considered by Sacks (68, 69), and has been reviewed by Sacks (70), Meyerhof (50, 53) and Cori (15, 16).

As in the case of mammalian tissues, there has been disagreement as to the mechanism of fermentation in microörganisms. The fact that a phosphorylating mechanism does exist in many microörganisms has been amply demonstrated by a number of observers, particularly Werkman and co-workers (82). However, certain observations have raised doubts as to the general application of the Meyerhof cycle in all microörganisms.

Fisher (19) stated in 1895 that fermentation of disaccharides or polysaccharides always involves preliminary hydrolysis. Willstätter and Oppenheimer (85) and Willstätter and Steibelt (84) challenged this view when they found that certain yeasts were able to ferment lactose and maltose more rapidly than the constituent monosaccharides. They furthermore observed that the fermentation of the disaccharides is not correlated with the content of the appropriate hydrolytic enzymes of the particular yeast. Similar results were obtained by Sobotka and Holzman (73). Wright (87, 88, 89) has more recently observed similar phenomena with respect to the fermentation of sucrose and lactose by

Streptococcus thermophilus, while O'Connor and Nord and Engel (63, 62) have suggested that *Fusarium lini* Bolley ferments trehalose directly. Leibovitz and Hestrin (36) have investigated this problem by comparing the rate at which maltose and methyl-α-glucoside are fermented by yeast and have come to the conclusion that maltose is fermented directly.

The mechanism of this phenomenon has not been satisfactorily explained. No phosphorylated forms of the disaccharides mentioned above have been isolated. It would seem, however, that a possible explanation of these data would be the direct conversion of the disaccharides to two molecules of hexosemonophosphate, in a manner analogous to the phosphorolysis of glycogen by phosphorylase. The hexosemonophosphate could then be broken down by the usual series of reactions. A reinvestigation of this problem from such a point of view should merit consideration. This would be in accord with the findings of Baba (5, 6) that phosphoglyceric acid is an intermediate in the fermentation of maltose by yeast.

The presence of enzymes in moulds capable of metabolizing carbohydrates without phosphorylation seems likely. Examples can be found in the work of Müller (54, 55) on glucose oxidase of *Aspergillus niger*, the formation of citric acid by *Aspergillus niger* (33), and the production of kojic acid by *Aspergillus tamarii* Kita (26).

Additional information on mould metabolism has been covered in the reviews by Tamiya (78), Raistrick (66), and Benhauer (8).

An attempt has been made critically to evaluate the evidence for the existence, particularly in animal tissues, of pathways of carbohydrate metabolism other than the Meyerhof cycle. The Meyerhof cycle is the result of numerous studies on extracts of muscle and yeast. The analysis of the reactions of the cell has necessarily been approached by a study of the presence and properties of enzymes that can be obtained from the disrupted cell. This has been done in most cases with a full realization of the limitations of such studies.

Studies of energy relationships, the action of inhibitors, rates of reactions, and the extractable enzyme components of tissues present a picture of possible reactions within the cell, but lead to only probable conclusions as to the course of these reactions within the cell. Since, however, a tremendous weight of evidence has accumulated favoring the existence of the Meyerhof cycle, the likelihood of its component reactions playing some important part in the degradation of carbohydrates within the cell seems very great. Therefore it becomes essential to determine whether any evidence is available to indicate that this series of reactions is not of primary importance within the cell. On the basis of the analysis presented in this paper there is at the present time little evidence pertinent to animal tissues which requires the assumption of a radically different scheme of glycolysis. The evidence for non-phosphorylating glycolysis is even less convincing.

In the case of microörganisms, suggestions of alternate pathways of glycolysis have a somewhat greater experimental basis.

The explanations offered in the review have for the most part depended on the

recent work of Meyerhof and Ochoa on the necessity of phosphate acceptors, the work of Cori and co-workers on mechanisms of phosphorylation, the work of Warburg and Christian on enolase and fluoride inhibition, and the work of Stickland on glyceraldehyde. It should be remembered that such explanations are suggested to indicate that the evidence does not require the acceptance of a new series of reactions to explain glycolysis in tumor, brain, and embryo. In many cases considerably more experimental data are required to prove the validity of such interpretations.

Thus the evidence at present does not rule out the possibility of the existence in animal tissues of glycolytic pathways other than the Meyerhof cycle, but the postulation of such alternate pathways is at present unnecessary to explain the facts.

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INFLUENCE OF ESTROGENS AND ANDROGENS ON THE SKELETAL SYSTEM

W. U. GARDNER AND CARROLL A. PFEIFFER

Department of Anatomy, Yale University School of Medicine, New Haven, Conn.

Sexual differences in the extent and form of osseous growth have been noted in many species, and in some species cyclic changes in the gross and microscopic structure of at least parts of the bones have been found to be associated with reproductive phenomena. This review will deal with the nature and extent of the influence of gonadal hormones on skeletal development and the studies pertaining to the mechanisms governing these changes. In connection with the latter studies it will also be necessary to consider the influence of sex hormones on the metabolism of bone salts. The great difference in the responsiveness of animals of different species has made it desirable to present this review more or less in phylogenetic arrangement.

INFLUENCE OF SEX HORMONES ON CALCIUM AND PHOSPHORUS METABOLISM. The changes in the levels of blood calcium and phosphorus which accompany egg laying and estrogen production have recently been reviewed (1). However, since many of these changes also affect the skeletal system they will be reviewed here to the extent to which they bear a relationship to, or afford an explanation of, the mechanism governing those osseous changes which are under the influence of the sex hormones.

Calcium metabolism—fish and amphibia. Hess et al. (2) showed that during the period when the eggs develop and mature in females of the cod and puffer fishes, the blood calcium level rises to 29 mgm. per cent as compared to 9 to 12.5 mgm. per cent in males. In the viviparous dogfish shark, on the other hand, no change in calcium is associated with the reproductive state. Again, in the toad Xenopus (3) the level of calcium in females is higher during the egg laying season than during the non-breeding season and at all times is higher than in males. Castration effectively lowers the calcium values in females and, after a prolonged period, in males. Ovarian extracts cause a rise in the blood calcium level in castrated or hypophysectomized females. Unfortunately the skeleton has not been studied. It was thought that the hormone-like action on calcium is exerted by some principle other than the sex hormones (3, 4). However, more recent investigation on other species has indicated that estrogen is the responsible hormone.

Aves. The most significant observations on calcium metabolism have been made on birds, as would be expected, since enormous amounts of calcium are excreted in the egg shell (5.5 grams of calcium carbonate in 16 hrs. or 40 mgm. of calcium per min. during certain periods while the shell is being laid down) (5). The pigeon is an excellent animal on which to study the correlation between increased serum calcium levels and egg laying (6) since the nature of its laying habits makes it possible to predict with accuracy the stage of development of the ovum at any given time. Two egg yolks are ovulated and then the ovary enters

upon a prolonged inactive period. During the period of rapid growth and ovulation of the two ova the serum calcium increases from 9.3 mgm. per cent, the value for sexually inactive females, to 19.9 mgm. per cent. Since the calcium level begins to rise 123 hours before the secretion of the first egg shell, it is obvious that the increase in serum calcium is not caused or explained by shell secretion. The rise is correlated only with the development of the two ova. Similar differences also occur between the serum calcium levels of non-laying and laying hens (7, 8) and are correlated with ovum size (9, 10, 11). Attempts have been made to show that the fluctuations which occur in the laying hens are due to a mobilization of calcium in the blood stream when an egg is not in the shell gland and a lowering of the serum calcium level when the egg is in the shell gland (5, 12), but the variations found were too small and inconsistent in trend to be very significant (13, 14). However, evidence, acquired by determining the ratio of CaO to P_2O_5 stored and excreted, indicates that calcium can be deposited in bone and from this reservoir be mobilized for formation of the egg shell (15, 16, 17). The amount that can be mobilized has been variously estimated as from 10 per cent (18) to 25 per cent (17) of the total calcium stores of the body. Mobilization of calcium from the skeleton is rapid but not as rapid as is the transference of ingested calcium carbonate to the oviducts (17). Therefore, if sufficient amounts of calcium carbonate are ingested, the shell gland is supplied from this source (19, 20), but if the calcium carbonate intake is low, calcium is withdrawn from the bones (15, 17). In the pigeon, however, it has been shown by careful histological study (21) that there is some destruction of medullary bone at the time of egg shell formation even though sufficient amounts of calcium are available in the diet.

In the non-laying hen the mesenteric vein contains the same level of serum calcium as does the mesenteric artery, but in laying hens the calcium in the vein is appreciably greater than in the artery (19). This increase in the amount of calcium transferred from the gut to the blood stream is evidently controlled by estrogen since it occurs only during the period of ovarian growth and egg laying. If calcium is withheld from the diet of the laying hen, egg laying ceases after about 12 days and the amount of calcium in the egg shells decreases to less than one-fourth the normal amount, indicating that only a limited amount of calcium can be withdrawn from the body stores (bones) for the production of shells (13). It has already been shown that the calcium brought by the blood to the oviduct is in a form which is different from that deposited in the egg shell (22). The extreme fluctuations in the serum calcium level which take place in the bird can be tolerated because the diffusible calcium remains constant, and only bound or non-diffusible calcium increases (23, 24, 25, 26). This hypercalcemia bears no relationship to the hypercalcemia observed in mammals which results from hyperparathyroidism or administation of parathyroid extract (27). Parathyroid extract does not cause a rise in blood calcium or a depletion of body calcium in non-laying hens, moulting hens, immature pullets, or cockerels (28, 29).

Influence of different estrogenic chemicals. Theelin, theelol, estradiol and estradiol benzoate increase the serum calcium level in pigeons (27, 30, 31), and

theelin, estradiol, estradiol benzoate and diethylstilbestrol are effective in this respect in chickens (32, 33, 34, 35). Likewise estradiol benzoate is effective in the duck (36) and the sparrow (37). Only Marlow and Koch (38) and Marlow and Richert (39) have reported negative results from the injection of estrogenic compounds into chickens, but it seems probable that they were using inadequate amounts of estrogen, since the doses they employed are too low to induce complete development of the oviducts.

Phosphorus metabolism. A great rise in blood lipid phosphorus levels occurs in apparently all oviparous vertebrates during the breeding season or following estrogen injection. This rise is presumably associated with the formation of the egg yolk. There is also a phosphoprotein (serum vitellin) which comprises an important part of the increased total phosphorus in breeding females of birds (40, 41, 42, 43), reptiles and fishes (40), but is found only in traces, if at all, in males and non-breeding females of these species. Unfortunately amphibia were not tested. Serum inorganic phosphorus, the only phosphorus compound associated with the metabolism of bone, has been followed rather incidentally to the studies on calcium and bone changes. It seems to be relatively less affected by the reproductive state of the animal or by sex hormones than are the lipid and protein phosphorus compounds. However, the intimate relationship of the serum inorganic phosphorus to the latter compounds makes interpretation difficult.

Amphibia. In the lower vertebrates inorganic phosphorus has been studied only in the toad Xenopus where, in contrast to calcium, it is decreased during the breeding season, and after injection of progesterone and estradiol benzoate (44, 45). However, the same author (44) reports that gonadectomy has no effect on plasma inorganic phosphorus.

Aves. In connection with the studies on the relation of calcium metabolism to egg shell formation in chickens it was noted that more P_2O_5 was excreted than ingested, indicating that calcium salts were being removed from bone for egg shell formation (15), and that the associated phosphorus was being released into the blood stream and excreted. This condition is similar to that found in hyperparathyroidism, but it is doubtful whether such a calcium-phosphorus relationship is also the explanation of the increase in serum inorganic phosphorus which occurs during the time bone is being deposited under the influence of injected estrogen. Small, probably inadequate, doses of estrogenic extracts have been reported to be ineffective in altering the serum inorganic phosphorus of chickens (38), but larger doses of estrogens raise the inorganic phosphorus in chickens (32), in ducks (36) and in pigeons (27).

Phosphatase activity. The serum phosphatase value in chickens (46) increases from 88.7 Bodansky units at one day of age to 114.0 at 10 or 12 days. It then gradually falls without showing any sex differences until the approach of the adult condition. In pullets the serum phosphatase value is 3.5 Bodansky units while in older non-laying hens it is 13.1 and in laying hens 17.1. In the mature cock the serum phosphatase value remains at 4.7. The relation of serum phosphatase to injected steroid hormones has not been studied.

INFLUENCE OF SEX HORMONES ON SKELETAL STRUCTURE IN BIRDS. *Pigeons.* During the course of investigations on bone marrow Kyes and Potter (47) discovered that at certain times the marrow cavities of female pigeons became so filled with spicules of bone that it was no longer possible to obtain marrow from them, but this phenomenon was never encountered in male pigeons. These authors further observed that the condition of the bones in the females was correlated with the size of the ovarian follicles. When the follicles were 2 mm. in diameter, or less, the femurs contained large marrow spaces. Osseous tissue partially invaded the marrow when the follicles were more than 4.5 mm. in diameter, and the marrow cavities were filled with bony spicules when the follicles were 10.0 mm. in diameter, or larger. Shortly afterwards Riddle and Dotti (30) demonstrated for the first time that injected estrogens cause a rise in serum calcium such as occurs normally at the time the ovary contains large ova. It was then but a short step to show that both hyperossification and the increase in blood serum calcium in pigeons can be produced by injected estrogen (31). It was found that with daily injections of 33.3 μg. of estradiol benzoate hyperossification began within two weeks after treatment was started and reached a maximum within five weeks, but it did not exceed that found in the normal female pigeon just after ovulation. Bloom, Bloom and McLean (21) made a careful study of the histology of the bone changes which occur during the normal egg laying cycle of the pigeon and found a very close correlation between the size of the ovarian follicles and medullary bone formation. An intense production of medullary bone occurs during the time of development and maturation of the follicle in the ovary and is followed by a period of intense destruction of medullary bone which coincides with the time the shell of the first egg is being formed, and continues for a few hours after the first egg is laid. Bone formation then begins once more and continues until shortly after the second egg reaches the shell gland. Again bone is resorbed while the second egg is in the shell gland and resorption continues at a diminished rate after the second egg is laid until all of the medullary bone disappears (about 10 days after the second egg is laid). The pigeon lays only 2 eggs in a clutch. During the bone formation phases, the reticular cells of the marrow become osteoblasts and these in turn become osteocytes. During the destruction phases the osteoblasts and freed osteocytes become osteoclasts, which, during the period of bone formation occurring between the laying of the two eggs, may again become osteoblasts. During the final postovulation period both osteoblasts and osteoclasts become reticular cells once more. With an adequate intake of calcium and phosphorus, formation of the organic bone matrix and calcification occur simultaneously, and during the periods of rapid bone destruction both are removed simultaneously. The bone formation associated with follicular development takes place in the granulocytopoietic portions of the marrow. Blood vessels are not encroached upon, and erythropoiesis is not appreciably affected. However, myelocytopoiesis practically disappears from the marrow at this time.

It was fortunate that Pfeiffer and Gardner (31) used sexually active male pigeons in their experiments on the relation of estrogen to hyperossification, since

Bloom, McLean and Bloom (27) discovered that doses of estradiol sufficient to induce medullary bone formation in male pigeons during the breeding season are ineffective in castrates or in males with inactive testes. However, the simultaneous administration of testosterone propionate and estradiol caused the formation of medullary bone with as little as 5 μg. of estradiol per day. The maximum effect of testosterone propionate in the combined treatment was reached with 1.0 mgm. daily. This synergism between male hormone and estrogen suggests a possible function for the appreciable amounts of male hormone produced by the ovaries of birds, studied particularly in the sparrow (48). The initial "endosteal reaction" may follow estrogen injection alone and consists of the deposition of a few lamellae of endosteal bone. Apparently androgen must also be present to bring about the accelerated endosteal proliferation with rapid formation of medullary bone which occurs in the normal osseous cycle. However, the administration of estrogen and androgen in any combination yet tried has not proved as effective in bringing about hyperossification as is the pigeon's own ovary. Hyperossification takes place at a much slower rate when experimentally produced than when occurring during the sexual cycle. Medullary bone formation may occur with an amount of estrogen which is relatively ineffective in raising the serum calcium level, especially when androgen is administered simultaneously, and androgen does not augment the calcium values found with a low dose of estrogen. Therefore, hyperossification and serum calcium level are not necessarily physiologically interdependent. The intramuscular injection of parathyroid extracts into birds in which medullary bone formation had been induced results in some resorption of bone accompanied by osteoclastic activity. Resorption does not, however, reach the proportions observed in the laying pigeon. It seems probable that the mechanism responsible for bone formation in the normal female pigeon has not been completely elucidated (27).

Chickens. Sexual dimorphism, while not evident in pigeons, is marked in fowl. This dimorphism is quite definite in the skeleton. Hutt (49) showed that a statistically significant sex dimorphism occurs in the mean absolute length of all bones of the appendicular skeleton of the chicken, the longer bones having a 13.4 per cent greater length in males than in females. The increase in length is not constant in all long bones but varies from 6.8 per cent to 16.5 per cent. Some evidence has been advanced that capons have slightly larger bones than do normal males (49), but very carefully controlled experiments indicate that castration of male chicks at hatching does not produce any changes in absolute or relative length of bones of the extremities in either normal or creeper fowl (50). That large doses of estrogen dwarf the bones of fowl is evident from the findings of Zondek (51) to be discussed later. Asmundson et al. (52) have also reported that excessive production of endogenous estrogen in young pullets following administration of pregnant mare serum dwarfs the tibias and tarsals. However, studies on gynadromorphism in the fowl (53, 54) indicate that the size differences in the skeletons of male and female birds is genetically, rather than hormonally controlled, since in the gynandromorph the skeleton may be of the male size on

one side and of the female size on the other, yet the hormone action should be the same on both sides. Other skeletal features, such as the width between the pubic bones, are distinctly different in the two sexes and are presumably affected by estrogen (52, 55), although accurate measurements seem to be lacking. Even the most accurate measurements failed to show a correlation between skeletal size and egg laying capacity within the limits of any one breed of chickens (56).

In connection with the stunting of growth which follows injections of large doses of estrogen (1,600,000 m.u., or approximately 533.3 mgm. of dimenformon in 16 weeks) Zondek (51, 57) observed that in x-ray plates of dwarf cocks produced by this treatment the opacity of the medullary cavity was the same as that of the bone surrounding it. With injections of 50,000 m.u. (approximately 16.66 mgm.) of dimenformon, the first indication of the induction of bone changes could be observed after 4 weeks, if treatment was started at the age of 6 weeks. When the bones were split longitudinally the medullae were found to be filled with spongy bone. The femur showed a reduction of 18.9 per cent in length and of 40.6 per cent in weight, while the tibia was reduced 30.8 per cent in length and 30.0 per cent in weight. However, Landauer et al. (35) showed by means of estrogen injections into sexually mature cocks, where no stunting resulted, that the hyperossification was not a result of the stunting but, as in the pigeon, occurred in response to the presence of the estrogen. Three weeks of treatment with a total of 1.19 mgm. of estradiol benzoate resulted in the deposition of a thin layer of new bone on the endosteal surface of the femur and tibia. This is an appreciably lower dose of estrogen than used by Zondek, especially when considered in terms of the body weights of the birds, but is much greater than that required for hyperossiffication in the pigeon. Higher doses of estrogen resulted in more extensive hyperossification. The effects of simultaneous administration of estrogen and androgen have not been tested. Therefore, it is not known whether androgen augments the formation of medullary bone in the chicken. Histological studies of the bone changes in the chicken have not been published, but it is assumed that they are quite similar to those taking place in the pigeon.

Other birds. Bone changes similar to those occurring in pigeons have been observed in ducks (36) and sparrows (37) following administration of estrogen. Large doses of estradiol benzoate are required to bring about these effects in the sparrow. In fact, the first attempt to obtain hyperossification in the sparrow was unsuccessful in spite of the fact that osseous changes occur in the normal female sparrow during the sexual cycle just as readily as in the laying pigeon (58). This failure has been attributed to the small doses used. Unfortunately all males tested were juvenals so that any effect an active testis might have was not observed. Evidence that androgen neither enhanced nor inhibited the degree of osseous change resulting from the administration of estrogen has been advanced by Pfeiffer et al. (37). However, these findings should be reinvestigated in the light of the observation that androgen augments the formation of medullary bone in pigeons (27).

The extent of new bone formation differs greatly in the two breeds of ducks which have been tested. Administration of relatively large doses of estradiol

benzoate (2 mgm. daily) to Mallard drakes, a low egg laying breed, resulted in the production of only a limited amount of medullary bone, while the same amount of estrogen administered to Pekin drakes, a relatively high egg laying breed, produced extreme bone changes. However, this material is too limited to give more than an indication that the differences in egg laying capacities influence the response to estrogen (36).

EVIDENCE INDICATING THE EFFECTS OF INTRINSIC SEX HORMONES ON THE DEVELOPMENT OF THE MAMMALIAN SKELETON. *Influence on general skeletal development.* The obvious differences in the sizes of the skeletons of males and females of many mammalian species have been undoubtedly associated with the influences of the gonads for many years. In addition specific sexual dimorphisms of parts of the skeleton such as the pelves show special sexual characteristics which may be in part genetically and in part hormonally conditioned. Although studies on sexual dimorphism and on the influence of gonadectomy upon the skeleton have infrequently been sufficiently extensive to prove the extent of humoral gonadal control of skeletal morphogenesis, such observations have contributed significantly to our information. Certain aspects of these investigations, especially of those pertaining to man, have not been covered completely in this review, but only sufficiently to indicate the trend and some conclusions of this investigational work. A few studies have been given more space than they may merit, but reference frequently is made to them in spite of the very limited material upon which conclusions were based.

Influence of castration in man. The Scopecs have afforded material for the study of the effects of castration on skeletal growth (59, 60). Among the Scopecs some individuals castrated before the age of puberty had abnormally longer appendicular skeletons and smaller calvaria than other individuals of similar heights from the same racial groups. Individuals castrated after puberty tended to resemble in stature the intact males of their racial group (61, 62).

A very detailed study was made on 31 eunuchs from North China (63). Nine eunuchs were castrated when less than 15 years of age. These eunuchs attained heights ranging from 163 to 182 cm. and averaging 172 cm. The heights of the individuals castrated when over 15 years of age ranged from 154 to 168 cm. and averaged 163 cm. at the time they were measured. The average heights to the tops of the symphysis pubes were 92 and 84 cm. for the early and late castrate groups respectively. The greater part of the difference in height therefore could be accounted for by differences in length of limbs. The eunuchs were also taller than other individuals from the same region.

Time of appearance and union of ossification centers in man. Pryor (64–67) reported that centers of ossification in females appeared at earlier ages than in males. His studies convinced him that the ossification of the skeleton of the female was always in advance of the male from the earliest appearance of centers of ossification; at first days, then weeks, months and years in advance. Detailed studies were made on the ossification centers of the hand (66, 68). Other investigators did not find the striking sex differences in skeletal maturation described by Pryor (69–72). Recently complete studies have been made on the

sequences of osseous development of the hand (70, 71, 72). Although Todd (72) observed a somewhat more rapid skeletal maturation in females he considered it closely related to the progress of sexual maturation, only negligible at the ninth year and not striking until the thirteenth year and from then on until the later teens. The stage of development of the skeleton of boys was associated more closely with evidences of sexual development than with the chronological age (73).

Some evidence exists for the early cessation of growth in cases of pathologically precocious hypergonadism; although growth may be accentuated, at least, for a while (74, 75, 76). Later, mention will be made of retarded growth (77, 78) and delayed union of epiphyses in hypogonadal patients.

Dogs. The skeletal development of an ovariectomized dog was compared with that of an intact littermate (79). The operation was performed at the age of 3 months and the animals were killed 12 months later. The ovariectomized dog was larger throughout the experiment, the bones of the extremities were longer, some of the epiphyses had not united and the pelvis was smaller than in the control. Significant differences existed between the males and females in the various indices and measurements of the heads of several breeds of dogs (80). The material, however, did not prove that these differences were attributed to the intrinsic gonadal hormones.

Sheep. At birth the skeletal development of male and female lambs was comparable (81). At the end of 5 months the bones of males were larger than those of castrate males, or of females. The bones of the head, neck and pectorial girdles of males were disproportionately large when compared with the females or castrates. Although the bones of the male pelves increased more in weight the relative increase was greater in the female.

Cattle. The measurements obtained by Tandler and Keller (82) showed that the skeletons of bulls and cows differed greatly and that the skeletons of castrated males and females were similar and of an intermediate type.

Mice. The formation of centers of ossification and the fusion of the epiphyses have been reported in mice but reference was not made to sex differences. The changes of the bones during the late developmental period were apparently similar in the two sexes. Histological studies were made on the distal ends of the femurs and proximal ends of the tibias of mice of seven different strains and from 1 to 30 months old (84). The epiphysial cartilages of the multiparous females became very thin and the resorptive phase began at an earlier age than in males or virgin females. The rapidity of changes with ageing lagged in males, during the first year of life being 2 to 3 weeks behind the females, but when resorptive processes became accentuated during the second year of life they progressed rapidly and soon the bones showed more resorption than those of the females. The differences in the rate of the ageing process in bones of mice of different inbred strains were apparently much greater than between the two sexes within the same strain.

"Breaking strength" and chemical composition. The "strengths" of the bones of male and female inbred C_3H strain and of hybrid mice at progressive ages did not entirely corroborate the above observations (85). The bones were strongest

in mice from 100 to 300 days old and thereafter decreased progressively. The femurs of the females were stronger than those of the males in almost all groups. Virgin females had stronger bones than the multiparous animals. The strength of the bones was associated directly with the density of the bone as determined by their radio opacity. The per cent of ash and the calcium phosphorus ratio were higher in the femurs of females than of males (86).

Rats—Appearance of ossification centers. The centers of ossification of the limbs of rats appeared at an earlier age in females than in males. The cleared limbs from large series of rats from 62 different litters were stained with alizarine and the number and size of the centers of ossification recorded (87). Littermate brothers and sisters were compared because the stage of ossification differed considerably between different litters of presumably the same age. Difficulties in the determination of chronological age or differences in environment were assumed to be responsible for the variations among different litters. X-ray examination of intact male rats and male rats castrated at one day of age revealed no effect of castration on the age of appearance or fusion of ossification centers other than the ischial epiphysis and os priapi which were delayed in the castrated animals (88).

Gonadectomy on skeletal growth. Castrate and intact male rats from the same litters attained similar weights (89) although ovariectomized females were 3.4 per cent longer and 23.5 per cent heavier than their unoperated sisters (90). Male rats castrated when 21 days of age attained adult weights 24 per cent less and had bodies 7.9 per cent shorter than those of the unoperated controls. The tails of the castrated rats were only 3.1 per cent shorter than those of the controls (91). Male rats castrated when 21 to 23 days old had shorter bodies and femurs than those of their intact controls when 88 to 105 days of age. The lengths of the tails and humeri of control and castrated groups were similar (92). Male rats castrated when 22 days of age and killed at 80 days of age were 10.9 mm. shorter nose-anal length and 26 grams lighter than their intact controls (93). Rats castrated at weaning and killed after one year had significantly shorter femurs, tibias, humeri, radii and ulnas than unoperated controls (94).

Spayed rats had longer bodies than hysterectomized controls (95). Although castrated female rats were 19 per cent heavier than their intact controls their body lengths and the lengths of their femurs were similar at 184 days of age (96). Rats castrated when 26 days of age had significantly longer femurs, humeri, bodies, and tails when 70 to 90 days of age but not when 184 days old (97, 98). The rats ovariectomized when young grew more rapidly for a few months but within six months were again no larger than the controls. X-ray examination of the skeleton revealed "longer bony structures" in castrated females than in intact controls (99). Female rats castrated when 40 days of age weighed more when from 80 to 290 days old than virgin females or females on normal or "forced breeding" schedules. The lengths of the tibias of the castrates also were greater during the first few months after castration but were eventually shorter than those of females from which the young were removed at birth. The virgin females were smaller and had shorter tibias than any of the other groups (100).

Sex differences on chemical composition. The per cent of calcium in the bodies

of female rats from 15 to 243 days of age was greater than in the males. The difference became definitely significant in animals over 30 days of age and was greatest in sexually mature animals. The absolute amount of calcium was greater in the males because of their larger size (101). Femurs of female rats from 23 to 150 days old had a higher ash content than did those of littermate males (102). The per cent of calcium and phosphorus was also higher in the bones of the females (103). The greatest relative change in the per cent of ash content per unit of age occurred in females at ages ranging from 20 to 30 days and at about 36 to 40 days in the males. These periods of rapid increment of ash were associated with phases of gonadal development (104).

Guinea pigs. Castration on length of bones. Male and female guinea pigs gonadectomized when 15 to 30 days of age and killed when 360 days old had body weights similar to those of the controls. Although both the gonadectomized males and females had shorter bodies, the long bones of the legs were nearly equal to or longer than those of the controls (105). These experiments in part confirmed earlier work by Bouin and Ancel (106) who found that the femurs and tibias were longer in prepuberally castrated guinea pigs. On the other hand castrated females bearing testicular grafts attained the size of normal males (107).

The tibias, ribs and joints of young gonadectomized male and female guinea pigs were obtained at weekly intervals and were studied histologically and compared with sections from the bones of intact controls (108). The ratio of hypertrophic to proliferating columnar cartilage cells was 4 or 5:10 or 11 for the male and female controls; 5:16 or 20 for the castrated males and 5 or 6:17 or 18 for the castrated females after five weeks. The epiphyseal cartilage thus increased in thickness in both groups of castrated animals. With degeneration of hypertrophic cartilage cells some calcification of the bars of matrix followed and ossification occurred. The cartilages of the joints also hypertrophied, tending to parallel the changes occurring in the epiphyses. The increased proliferation of cartilage occurring during the first months after ovariectomy eventually ceased and sclerosis and ossification progressed although both at a slower rate than in intact females (109). Gigantism did not occur, although maturation of the osseous system was delayed.

Influence on the dimorphism of the pelves. The shapes of the pelves in man differ in different individuals—especially the shapes of the pelvic outlet. The pelves of children of under 11 years of age were almost all of the anthropoid or dolicopellic type as determined by the roentgenological method of Thoms (110). The pelves of the males and females began to assume their specific sexual characteristics in children approaching fifteen years of age. The observation on 59 female and 16 male children from 5 to 15 years of age thus "suggested that both males and females start out life with pelves which are identical in type and that the major differences observed in adult male and female pelves do not appear until after puberty and are therefore due to the influence of sex hormones" (111). Similar observations had been made by Odiditsch (112), who also noted that chronic disease modified the pubertal metamorphosis of the pelvis.

The types of pelves of both males and females ranged widely from dolicopellic

to platypellic but the pelves of males showed 1, more prominent and heavier ischial spines; 2, narrower pubic angles, and 3, narrower greater sciatic notches than those of females. The pelves of the "more masculine" or "more feminine" individuals did not necessarily show the most accentuated characteristics of the respective sexes. In fact two hypogonadal males had typically masculine types of pelves. The morphological characteristics of the pelves were not necessarily a reliable indication of the degree of maleness or femaleness of the individuals possessing them (113, 114).

During pregnancy the pelves enlarged slightly by a loosening of the sacroiliac ligaments and the pubic symphyses. A joint-like space was frequently encountered in the symphyses of females (115, 116, 117). The pelvic loosening may be of sufficient magnitude to impair locomotion because of lack of pelvic rigidity. Pelvic mobility, when once it has appeared, may recur with each subsequent pregnancy. Repair may occur following delivery (118, 119).

Sheep. The pelves of newborn lambs showed no sexual dimorphism. In the adult male the pelvis was larger than in the female, composed of heavier bones but longer and narrower than in the female. The pelves of castrated males and females were smaller than those of the females but were similar in shape. True pelvic sexual dimorphism existed and was determined, apparently, by the sex hormones. The castrate type, however, more closely resembled the pelvis of the female (120).

Guinea pig. The bony pelvis has been associated more frequently with reproductive function than have other parts of the skeleton. The guinea pig's pelvis apparently attracted the attention of investigators because of the large size of the young at birth. The separation of the pubic bones of the guinea pig before parturition has been described repeatedly (121, 122, 123).

Virgin guinea pigs had distinct pubic symphyses (124). A chondrofibrous junction of the pubes appears in young virgin female guinea pigs, the amount of fibrous tissue increasing at the expense of the cartilage forming a syndesmosis (125). Some cyclic separation of the pubes may recur with the repeated estrous cycles (126). The fibro-cartilaginous interpubic unions of male guinea pigs were replaced by bone in old animals so that the symphyses were partially destroyed.

During pregnancy the amount of fibrous interpubic tissue increased greatly, especially after the first five weeks (125). Subsequently some bone resorption of the symphysial face of the pubes occurred. The marrow spaces were exposed and the fibrous tissue of the symphysis showed increased vascularization. The proliferation of connective tissue cells was especially rapid during the last two weeks of pregnancy and the pubes became widely separated just before parturition. The interpubic ligaments were shortened directly after delivery but were never completely lost.

Pocket gopher. Two types of pelves have been described in each of several species of Rodentia and Insectivora but the author did not associate them with sex (127). The female type of pelvis of pocket gophers was associated with the presence of functional ovaries—the pelvic skeleton was a secondary sexual character (128). The dimorphism first became apparent at puberty and pro-

gressed with the complete removal of the medial part of the pubes. Orchidectomy did not alter the development of the usual male type of pelvis. Ovariectomy usually prevented the formation of the female type of pelvis.

Mouse. The pubes of male mice were united firmly by fibrous and cartilaginous tissue throughout life and the amount of interpubic cartilage decreased with advancing age (129). The pubes were less firmly united in adult virgin females, but the cartilaginous symphysial faces were closely approximated by ventral and dorsal ligaments which enclosed a joint-like space. During pregnancy the medial parts of the pubes were absorbed and interpubic ligaments formed. After one pregnancy the ligaments were about 2 to 5 mm. in length and became longer in mice having several litters. Following delivery the pelvic architecture never returned to the condition of that of the virgin female. The greater part of the interpubic ligaments formed during the latter 8 days in intact pregnant mice, in pregnant animals from which the fetuses had been removed but the placentas retained (130) and in hypophysectomized pregnant mice (131, 132).

INFLUENCE OF INJECTED SEX HORMONES UPON SKELETAL DEVELOPMENT. *Rats —effect of estrogens.* Several investigators have noted that the injection of estrogens inhibited or reduced the rate of increases in body weight. Fewer observations have been made on osseous or skeletal growth but there is general agreement that large doses of estrogens inhibit or stunt growth when injected over prolonged periods.

The skulls and long bones of estrogen-treated male and female rats were smaller (133). Twice weekly 500 I.U. of estradiol benzoate were injected into rats 4 to 6 weeks old and the growth of the treated rats was compared with the controls. After 3 to 4 weeks the growth increments of the treated rats decreased and ceased after 3 to 4 months. Body length was also greatly reduced (51). Later it was shown that these "eunuchoid dwarf" rats had skeletons more opaque to x-rays than the controls. The medullary areas were invaded by bone. The lengths of the bones were reduced (57, 134). Zondek assumed that estrogens inhibited not only the gonadotropic function of the pituitary but also the growth-stimulating function (134).

Theelin when injected into young rats at the rate of 20 μg. per day had no effect upon body length during experiments extending over 5 to 10 days, although body weight was reduced and the tails of the estrogen-treated rats were shorter than those of the controls (135). Rats receiving 20 μg. of estrone daily from the 21st to the 99th day weighed less, were shorter, and had shorter tails and femurs than the controls (136). The simultaneous injection of testosterone propionate (1 mgm. per day) prevented the decreased rate of gain in weight and length observed in estrogen-treated rats (137).

The daily injection of 12.5 μg. of estradiol to male and female rats from 1 to 7 or 9 days of age "causes a significant advance in skeletal age of females but not of males." Treated females had 32 per cent more centers of ossification in their appendicular skeletons than did the males (138).

The oral administration of 50 to 250 μg. of stilbestrol daily greatly reduced the rate of growth of young rats (139).

The daily injection of 0.2 to 1 mgm. of estrone inhibited the longitudinal growth

of the skeleton of young rats and decreased the thickness of the epiphysial discs. The daily injections of 200 μg. of stilbestrol were more effective in stopping osseous growth. The injection of hypophysial growth-stimulating hormone prevented the inhibiting action of estrone on the proliferation of cartilage and bone (140, 141). Since the epiphysial changes of the hypophysectomized and estrogen-treated rats were not similar it was assumed that the estrogens did not act by opposing the production of growth-hormone but acted directly upon the growth zone of the epiphysial cartilage (141). Growth hormone did not completely prevent the inhibition of skeletal growth in animals given large amounts of stilbestrol (142).

Histological changes. Several factors have been considered in microscopic studies on growing bones, namely, the proliferation of cartilage at the epiphysial junction, the sclerosis of cartilaginous matrix and the proliferation of the osteogenic tissues at different sites within or about the bone.

Young rats given large doses of estrogens for periods of 17 to 26 days had slightly thinner epiphysial cartilages and an increased number of osseous spicules in the proximal metaphyses of their tibias. The amounts of osseous tissue were greater in the females than in the males. Untreated control rats "pair fed" at the levels consumed by the estrogen-treated rats, had thinner epiphysial cartilages than the ad libitum fed controls but the cartilages were not as thin as in the estrogen-treated rats. The tibias of adult estrogen-treated rats showed similar but less extensive changes (143).

The bones of rats which had received weekly injections of 33 μg. to 83 μg. of estradiol benzoate from weaning age up to 17 months were studied by the Silberbergs (144). After treatment for 4 months the epiphysial cartilages were thin and partially sclerosed. The cartilages were sometimes absent in rats treated for 9 or 10 months. The latter condition was observed in some untreated rats 17 months old. The sub-epiphysial marrow-containing areas of the tibias of rats treated for 4 months were relatively fibrous, contained numerous osteoblasts surrounding an increased number of newly formed osseous trabeculae, some of which surrounded sclerosed spicules of cartilage. The apposition of bone had been excessive during this period. By the 9th month resorption of the osseous spicules had occurred so that the metaphyses of the bones resembled those of the controls.

A well illustrated report of the osseous changes in rats given 10 mgm. pellets of estradiol dipropionate weekly for periods up to 6 weeks has reaffirmed and extended a part of the above observations (145). After one week the epiphysial cartilages of young estrogen-treated rats were thin and the number of osseous trabeculae reduced; "the cartilage histology resembles that of an animal that had been almost starved," a probability since appetite was reduced. After 3 weeks the cartilage had partially recovered but was again reduced after 6 weeks. During the first 3 weeks many small spicules formed and later fewer coarse trabeculae of bone occupied the greater part of the proximal medullae of the tibias. The increased rate of ossification did not occur in estrogen-treated hypophysectomized rats (146).

The injection of estrogens was considered to induce localized osteofibrotic

lesions in young rats (147). This fibrosis occurred chiefly subperiostially near the metaphysis and was assumed to be responsible for the absence of "clubbing" of the bones or for the maintenance of their normal shape during longitudinal growth (148).

On composition. The calcium-phosphorus ratio of the bones of estrogen-treated rats was higher (2.39) than that of controls (2.10). This change in composition was attributed to a decrease of phosphate and an increase of carbonate in the bone salts since the total calcium was not increased (149).

Body weight was slightly lower but the weights and ash contents of the bones of very young rats which had received 50 to 250 μg. estradiol benzoate during 7 to 50 days were similar to those of the controls (150).

The femurs of young or young adult rats which had received estrogens for 44 days or more contained a significantly higher per cent of ash than those of their controls when they were fed an adequate diet (143). The femurs of estrogen-treated old rats showed no increase in ash during experiments extending over 37 days. In none of the experiments was the total ash increased significantly in the estrogen-treated animals because of the decreased growth of these rats. Estrogen-treated rats ate less than their ad libitum fed controls. "Pair fed" controls gained more weight than the injected rats but their femurs contained less ash and also a lower per cent of ash. The decreased food intake could account for neither all of the decreased gain in body weight nor the increased per cent of bone ash. Bone phosphatase increased significantly in rats treated for 17 days.

Repair of fracture. Callus formation occurred somewhat sooner about fractures of femurs of castrated rats given estrogen than in the untreated controls (151). Although the effects upon the fractures were slight the authors "gained the impression that injections of estrogenic substances stimulated the production of endosteal osteoid tissue," although the fractures in the different treated and control rats were at different and overlapping stages of repair (152).

Androgens—Size of bones. Androgens, especially testosterone and its esters, have been reported to either stimulate or inhibit body growth or growth of the skeleton. Factors such as the dose of hormone given, the duration of treatment, and whether or not the animals were castrated may influence the results. These factors will not account for all of the opposing results that have been reported. Daily doses of testosterone of 200 μg. did not alter growth in young rats treated for 1 or 2 months (153). Doses of 1400 μg. of testosterone propionate reduced the weight-increment in another group of young rats while smaller doses (167 μg.) stimulated growth (154). Young rats given 35 mgm. of testosterone propionate during 40 days gained much less weight and had shorter bodies, tails, femurs and tibias than oil-injected or untreated controls (155). The epiphysial cartilages were thin and atrophic. Testosterone propionate (1 mgm. per day) increased the rate of gain in weight and length of young rats and prevented the growth-inhibiting effect of estrone (137).

Male rats given 1 mgm. of testosterone propionate daily for 80 days weighed less and were shorter than untreated controls. Differences in weight and length were not apparent at the 26th day of injections but the controls were 6.5 mm.

longer at the 80th day (156). Castrated rats were on the average 11 mm. shorter than intact untreated controls and castrated rats given 1 mgm. of testosterone propionate daily were 13.5 mm. shorter. Again the differences were not apparent until at the 26th day (157). Castrated and intact rats given small doses of testosterone propionate (50 μg.) 6 times weekly increased in weight more rapidly than the controls (158). Although no measurements were taken the weight changes of young rats, some injected from the first day of life and given from 1 mgm. of testosterone propionate 3 times weekly to 5 mgm. daily, did not differ greatly from those of the oil-treated controls (159). Some of the experiments were continued for 25 weeks.

The os priapi and ischial epiphyses appeared much earlier in male rats given 0.25 mgm. of testosterone propionate daily or every other day but after 87 days there was no significant difference between the treated and control group (88). A systematic x-ray examination for the time of appearance and fusion of many other centers of ossification showed no difference between controls and treated rats. Doses of this size or larger (1 or 2 mgm. daily) did not alter body weight or skeletal maturation even when started at birth and continued from 16 to 135 days.

Upon os priapi. Many studies have been undertaken recently on the influence of sex hormones on intrauterine or newborn rodents. None have shown effects of hormones upon the skeletons of the young. Estrogens will apparently partially inhibit the development of the os priapi in fetal males (160). Castration immediately after birth did not prevent the development of the os priapi in the male but the os failed to develop in the masculinized clitoris of rats given small doses of androgen after birth (161). Large doses of androgen injected into the pregnant mother induced well developed os priapi in the intersex female young born under such conditions (162).

Mouse. Effect of steroid hormones on gross changes. Although the pubic bones were partially resorbed in estrogen-treated mice the density of the other bones of the skeleton was increased (129). The medullae of the long bones of mice which had received 3.3 to 33.3 μg. of estradiol benzoate or 0.1 mgm. of equilin benzoate weekly for prolonged periods contained either excessive numbers of fine or coarse osseous trabeculae or, after more prolonged treatment, compact bone (163). The progression of changes depended upon the amount of hormone used and duration of treatment. Similar gross skeletal changes were induced by injections of estrone, equilin, triphenylethylene, and stilbestrol (165). The bones of mice of some strains responded more rapidly than others.

The simultaneous administration of 0.625 to 2.5 mgm. of testosterone propionate completely prevented the excessive accumulation of medullary osseous tissue in the long bones of estrogen-treated animals (164, 166).

"Breaking strengths" and composition. The bones of estrogen-treated mice had a higher ash content (69 per cent) than those of control males (59 per cent) or females (64 per cent) and a higher calcium-phosphorus ratio (86). The ash content of the femurs of mice receiving testosterone propionate or estrogen plus testosterone propionate was 60 per cent, hence approached that of the normal

males. The new endosteal (medullary) bone apparently had a slightly higher calcium-phosphorus ratio than the bone of the diaphyseal shaft.

The structure of the bones of estrogen-treated mice differed from those of the controls as determined by x-ray diffractograms. The bone in the former medullary region was entirely unoriented and the diffractograms differed from those obtained from cortical bone or bones of untreated mice (167).

The femurs of estrogen-treated hybrid mice had "breaking strengths" of 1599 to 3542 grams (average 2499 grams), whereas femurs of controls of the same age groups had "breaking strengths" of 1244 to 2101 grams (average, 1655 grams). The average "strength" of the femurs was thus 844 grams greater in the mice which had received weekly subcutaneous injections of 16.6 to 50 μg. of estradiol benzoate (168). Similar observations have been made on a larger series of mice (85). The "breaking strengths" of the femurs of estrogen-treated males were greater than those of the estrogen-treated females. The differences in "breaking strengths" of femurs between the estrogen-treated and control groups tended to increase with advancing age. The estrogen tended to prevent the thinning of the diaphyseal walls associated with advancing age. The bones of the mice given testosterone propionate had very low "breaking strengths."

Histological changes. The bones of estrogen-treated mice became almost solid, or the medullae were filled with spicules greatly reducing the marrow. The apposition of bone was entirely endosteal—no exidence of excessive periosteal growth was observed (163–166).

A roentgenographic and histological study of the femurs of mice of two strains which had received weekly doses of 150 to 1000 r.u. of estradiol benzoate showed sclerosis of the cartilage and "osteoblastic activity" leading to generalized osteosclerosis. The long bones were first and most extensively affected but the vertebrae and calvaria also showed excessive osteogeneses and delayed resorptive processes (169).

Detailed and comparative histological studies have been made on the bones and articular cartilages of young and old estrogen-treated mice from several inbred strains (170, 171). The mice received different levels of hormone for different periods of time. Within 1 to 2 weeks after the injection of estrogens was started the epiphysial cartilages were reduced in thickness and the number of layers of hypertrophic and columnar cartilage cells was reduced. The cartilaginous ground substance became hyalinized and sclerosed. The reduction of the width of the cartilage and extent of hyalinization and sclerosis of cartilage matrix increased in mice treated for longer periods (3–6 weeks). With more prolonged treatment calcified matrix or bone separated the epiphysial cartilage from the marrow and in some areas the cartilage was completely eroded, exposing the marrow of the epiphysis and diaphysis.

The vascular marrow of the metaphysis became less vascular and increasingly fibrous (170). "Epithelioid" cells appeared in the fibrous marrow after treatment for 2 weeks. These cells then increased in number and "became converted" into osteocytes. New bone matrix was formed by these osteocytes forming first small and then larger spicules and finally a dense bony meshwork. Resorptive processes were largely inhibited during this time. After 2 or 4

months "osteoclastic resorption" set in and the bone was gradually removed until, at 9 months, few metaphyseal trabeculae remained. The diaphyseal walls were thickened during the first 4 to 6 months of treatment and thereafter were reduced gradually in thickness.

The extent of the bone changes was greater in the males than in the females. The bones of mice of some strains were affected to a much greater extent than those of other strains.

When the injections of estrogens (100 to 200 r.u. weekly of estradiol benzoate) were started in adult (6 to 11 months old) or in aged mice (12 to 18 mos. old) less or no increase in the thickness of the metaphyseal spicules or diaphyseal walls occurred. The age changes in the epiphysial cartilages were accentuated and the resorptive phases stimulated precociously rather than inhibited. Proliferative arthropathic lesions occurred less frequently than in untreated controls. Estrogenic treatment accentuated rather than inhibited the usual resorptive phases of the ageing process when the injections were started in old animals.

The proliferation of cartilage and resorption of bone were also inhibited in young mice given 0.25 to 0.85 mgm. of testosterone propionate weekly for 2 weeks or more (172). The changes were similar to those occurring in estrogen-treated mice but much less striking.

Guinea pigs. Guinea pigs which received 50 to 100 I.U. of estradiol benzoate daily for 27 to 38 days had thinner epiphysial cartilages than the controls; the layer of hypertrophic cartilage was especially reduced (173). The inunction or injection of estrogens decreased the rate of gain in weight and longitudinal growth of the tibias of young guinea pigs (174).

The daily injection of 20 r.u. or biweekly injection of 250 r.u. of estradiol benzoate into male and female guinea pigs weighing 140 to 190 grams increased the rate of hyalinization of the cartilaginous ground substance of the epiphysial discs (175). The amount of the hypertrophic cartilage first decreased and then increased while the proliferative cartilage showed an inverse relationship. Ossification and sclerosis of the cartilage were increased. Metaplasia of the columnar and hypertrophic cartilage cells to osteocytes was described. In the metaphyses "epithelioid" cells replaced the vascular marrow and osteocytes appeared and formed spicules of bone and abundant osteogenic tissue. Because resorption was reduced an increased amount of bone appeared in the metaphysis. The diaphyseal shaft was thickened and became denser during the first few months but later became thinner and more porous as resorptive processes became accentuated. Animals of both sexes responded similarly. The doses were not large enough to prevent growth although the estrogen-treated animals grew less than the controls (174, 175).

Estrogen-treated guinea pigs, one week old, when given 50 to 100 r.u. of estradiol benzoate weekly showed first a decrease in the thickness of the epiphysial cartilage and then an increase (175). The layers of both the proliferative and hypertrophic cartilage increased in thickness and the tendency for sclerosis of cartilage and osteoblastic proliferation and ossification were reduced appreciably.

Progesterone increased the proliferation of columnar and of hypertrophic

cartilage and inhibited sclerosis and retrogressive changes in cartilage when injected in the amounts of 0.65 to 1 mgm. 6 times weekly (176). Bone formation was relatively reduced "thus counteracting the aging of bones."

Dogs. Two young dogs treated with estrogen (Menformon) showed precocious union of the epiphyses (177). Puppies given 1.66 to 3.33 mgm. of estradiol benzoate weekly for 5 to 7½ months, weighed less and were shorter than untreated dogs. The epiphyses were united precociously. The pubic bones were not altered and in the males the growth of the os priapi was inhibited (178).

Man—Estrogens. Very few studies have been undertaken in man on the effects of administered estrogens on osseous growth or on bone salt retention. The daily injection of 2,000 I.U. of estrone to young girls for 6 to 18 days resulted in a depression of the calcium retention. The amount of hormone injected was adequate to cause mammary swelling and a vaginal discharge (179).

Albright and his associates have associated post-menopausal osteoporosis with a deficient production of estrogenic hormone. The post-menopausal osteoporosis most frequently involved the vertebra and the pelves; the long bones were rarely involved (180, 181). The injection of estrogen into such patients resulted in a positive calcium retention and marked subjective improvement. Nitrogen retention was also increased.

Androgens. The occasional occurrence of hypogonadism and retarded growth or of retarded genital and somatic growth probably indicate the desirability of the following investigations. Children showing retarded sexual development, sub-normal growth and a sub-normal bone age showed accelerated growth when gonadotropic hormones were injected (182, 183). Although the rate of growth was much in excess of that expected from standard growth charts the bone-age changes were not proportionally accelerated (184, 185, 186). The secondary sexual characters also usually developed indicating gonadal stimulation.

The possibility that the accelerated growth resulted from the androgenic activity of the stimulated testis was investigated by direct injection of androgens into similar patients. One 21 year old eunuchoid individual gained 4 cm. during the 6 months of treatment with testosterone propionate (10–30 mgm. daily) (187). The injection of testosterone propionate or implantation of pellets of the hormone lowered the age of epiphysial appearance in 1 young patient but did not greatly accelerate rate of union of epiphyses in adult hypogonadal individuals (188). Spurts of growth were observed in adolescent hypogonadal individuals given testosterone propionate but the rates of epiphysial closure were not sufficiently altered to be detected by x-ray examination (189). Accelerated increase in height has been reported in a eunuchoid patient given testosterone (190). Either testosterone propionate or methyl testosterone increased the rate of growth of hypogonadal individuals beyond that expected. Skeletal age, as determined by epiphysial union advanced more rapidly than might be expected from changes in chronological age (191, 192). The frequent occurrence of growth rates in excess of those expected from comparison with charts of normal growth was noted in hypogonadal boys given either gonadotropic hormones or testosterone. Bone-age was usually not altered greatly (193).

Patients showing post-menopausal osteoporosis showed increased retention of calcium and phosphorus when androgen was administered in combination with estrogens (182).

Influence of injected hormones on the pelves of guinea pigs. Hisaw (194) apparently first attempted experimentally to induce a separation of the pubes of virgin guinea pigs similar to that occurring at parturition. The pubes of some female guinea pigs became separated by an interpubic ligament when the blood sera of pregnant rabbits, dogs, and other animals were injected. The animals which responded were at or near estrus when the injections were made. Later the relaxation-inducing substance (relaxin) was found in the placentas and corpora lutea of certain animals (195) and was effective only in guinea pigs which recently had been under the influence of estrogens, either intrinsic or injected.

Several other investigators noted that estrogens would induce some ligamentous proliferation at the symphysis as determined by mobility of the pelvis and x-ray examination (196–199). Progestin was not effective by itself or when given with estrogens (196), although a non-estrus producing "extrait mobilisant" could be obtained from corpora lutea. Other investigators have observed an extensive separation of the pubes of young, intact or ovariectomized guinea pigs which received estrogens (estrone or estriol) for prolonged periods. Short periods of treatment with estrogens, alone or with progesterone, resulted in slight pelvic changes (200, 201). More recently estrogen and progesterone when administered simultaneously induced extensive pubic separation and the identity of "relaxin" as a specific hormone was questioned (202).

Although the chemical identity of "relaxin" has not been determined and some investigators have doubted its specificity of function, blood of pregnant laboratory animals and of women apparently contains a substance capable of inducing a rapid pelvic relaxation in estrous guinea pigs. Hisaw first detected the substance in the blood of pregnant rabbits, but not in women. Other investigators found that 3 to 6 cc. of blood sera from women during the first half of pregnancy would induce pelvic relaxation in approximately 75 per cent of estrous or adequately treated guinea pigs and that larger amounts were more effective (203, 204).[1]

The experiments on the relaxation of the pelvis of the guinea pig are not in agreement. Few investigators have claimed as rapid and/or extensive a pubic separation as occurs during pregnancy from any treatment with estrogens and progesterone. The presence in blood of pregnant animals, early pregnancy in man, and in aqueous extracts of corpora lutea of a relaxation-inducing substance (relaxin) has been observed repeatedly. These experiments indicate

[1] Recently Hisaw and his associates have added further evidence for the specificity of "relaxin". Although the pelvic ligaments of castrated guinea pigs would relax when large doses of estrogen and progesterone were given such changes would not occur in hysterectomized, castrated guinea pigs. Relaxin, on the other hand, was effective in hysterectomized estrogen-treated animals. Estrogenic or progestational activity could not be detected in animals given large doses of relaxin. References—Hisaw, F. L. et al., Anat. Rec. **84:** 7 abst. 1943; A. A. Abramowitz et al., Anat. Rec. **84:** 6 abst. 1943.

that the active material occurs commonly in pregnant animals and is not one of the known steroid hormones.

Mouse. Almost simultaneously two investigators reported that estrogen-treated mice showed a ligamentous separation of the pubes (205, 206). The interpubic ligaments were readily discerned upon x-ray examination of the carcasses or upon gross examination. The ligaments varied in length up to 7 or 8 mm. Estrone, estriol, estradiol, equilin and equilinen induced similar changes. The injection of estrogens thus resulted in a transformation of the pubic symphyses of males or virgin females to the type of interpubic connection found in multiparous females.

The interpubic ligaments were formed by a proliferation of the fibrous connective tissues of the ventral and dorsal fibrous ligaments and periosteum. Resorption of the medial parts of the pubic and ischial bones also occurred. When the injections were started in very young animals the pubes were sometimes largely resorbed. The injection of testosterone inhibited the pelvic changes when administered with estrogenic hormone (164). It likewise largely prevented the pelvic changes occurring during the latter part of pregnancy in primiparous mice (207).

Although quantitative studies on the sacro-iliac ligaments have not been made, the impression was that mobility was increased at this point. The parts of the innominate bones not resorbed showed a slight increased ash content as did the femurs (86). The shapes of the innominate bones of male and female mice differ from one another, the ischial angle being greater in the female. The injection of estrogens did not alter the male type of ischial angle, even when the injections were started in young animals (129).

INFLUENCE OF SEX AND OF STEROID HORMONES UPON SERUM CALCIUM LEVELS.

Rats. Parathyroidectomized or castrated rats showed no change in serum calcium levels subsequent to injections of 600 m.u. of theelin (209). On the other hand, the serum calcium levels of intact, hypophysectomized or gonadectomized rats were increased uniformly after the injection of several different estrogenic chemicals (30). Marked elevations of the levels of serum calcium were observed in estrogen-treated rats in another laboratory and in addition the amount of ingested calcium retained was increased during the first few days of observation and later decreased (210). The serum calcium levels of young rats after 6 to 12 daily injections of 100 I.U. of theelin were slightly elevated (211). The levels of serum calcium were slightly but significantly increased in rats which had received estrogens and in some groups the serum phosphatase was decreased (143). Other investigators have reported a slight increase in the phosphatase content of femurs of rats receiving estrogens and androgens (212).

The serum calcium levels of estrogen-treated rats usually increased above pretreatment levels. This increase may be slight but statistically significant (143, 211) or of greater magnitude (30, 210).

Rabbits. Adult rabbits injected with crude ovarian extracts presumably containing some active material, showed a slightly lower serum calcium within 24 hours (213). A rapid and rather marked decrease in serum calcium occurred

within a few hours after the injection of large amounts of estrone into spayed rabbits (214). Other investigators observed no significant changes in serum calcium in rabbits given extracts of ovaries (210, 215) or in rabbits which had received 100 μg. of estrone daily for 15 to 31 days (212).

Although high serum calcium levels have been observed in castrated rabbits (214) this observation has not been observed consistently (210).

Dogs. Dogs showed a slow but marked rise in serum calcium after ovariectomy (216). Slight seasonal variations in the levels of serum calcium were noted in female dogs. The higher levels were associated with the seasons during which estrus occurred (217). Two dogs which had received 300 r.u. of estrone daily for 7 days showed an elevation of 2 mgm. per cent of serum calcium (30). The total and diffusible serum calcium levels of other estrogen-treated dogs increased about 2 mgm. per cent and again returned to normal levels after the cessation of treatment (218).

During proestrum or estrus tetany developed in thyroparathyroidectomized dogs sometimes without appreciable changes in the amount of serum calcium (219, 220, 221).

Parathyroidectomized dogs given estrone or theelol showed a decrease in serum calcium and mild or severe tetany. Intact dogs showed no serum calcium changes after estrogenic treatment (222, 223). Tetany occurred during the onset of spontaneous estrus in parathyroidectomized dogs maintained on minimal amounts of vitamin D and calcium. The blood calcium levels of rachitic dogs decreased when theelin was injected (224).

Cattle. The serum calcium levels of male and female cattle at different ages and following castration were determined by Frei and Emmerson (225). Slight differences between the different groups were noted. During estrus the serum calcium level rose in some animals. The injection of small amounts of estrogen was followed by a decrease in serum calcium. Following the injection of large amounts (20.0 to 120.6 mgm.) of estradiol benzoate the serum calcium levels of lactating cows were lowered and the serum phosphatase and inorganic phosphorus tended to rise (226).

It is difficult to generalize on the action of estrogens on levels of serum calcium in mammals. The observations even within individual species are not consistent, and are more variable when different species are compared. Most of the studies have been deficient in that certain factors such as food intake and dietary regime were not followed. However, since the alterations in serum calcium in the mammalian species are so slight it is probable that, if anything, they merely reflect changes occurring in the osseous or other tissues concerned with the utilization, storage, absorption or excretion of calcium. There is as far as the reviewers know, no evidence that estrogens influence calcium metabolism in the same manner as do the parathyroids or the "vitamin D" compounds.

RÉSUMÉ

Oviparous animals which have oviducts that secrete or deposit calcified shells around their ova have an obvious need for some mechanism to co-ordinate certain

aspects of calcium metabolism with reproductive function. Since the bones act as storehouses for the calcium reserves of the body, these tissues might also be associated with reproductive functions. The drain upon the maternal calcium stores in mammals imposed by embryonic skeletal development or by lactation might indicate a need for some mechanism of interrelationship between reproductive function and calcium metabolism in mammals.

The injection of estrogenic hormones into birds increased the levels of serum calcium and accelerated the formation of endosteal bone. However, the rapid proliferation and ossification of osteogenic tissue could occur in estrogen-treated birds in the absence of marked changes in the levels of serum calcium especially when small amounts of androgen were also injected. The steroid hormones therefore may influence both bone proliferation and the mechanism regulating calcium levels, but these two phenomena are not necessarily related. In some laboratory rodents the sclerosis of cartilaginous matrix, proliferation and ossification of medullary osteogenic tissues were augmented in estrogen-treated animals, but the levels of serum calcium were unchanged or only slightly altered. Androgens prevented the excessive osseous growth when administered to mammals given estrogens.

At this time the reviewers believe that the augmented medullary proliferation of bone may be most satisfactorily explained by assuming that estrogens stimulate osteoblasts or the differentiation of esteoblasts from undifferentiated elements of the marrow. The apparent differences in the response of the osteogenic tissues of birds and mammals to androgens when injected simultaneously with estrogens may be due to some fundamental differences in the changes induced in the cells of the marrow.

The gonads or the steroid sex hormones regulate to some extent the morphogenesis of the skeleton and may control in part the extent of skeletal growth. Large amounts, especially of estrogens, inhibit the growth of cartilage and hence longitudinal osseous growth. The function of the pituitary gland is probably also altered in such animals and may be directly responsible for the ensuing "dwarfism." Small amounts of hormones, especially of androgen, may augment the rate of longitudinal growth of the skeleton.

The steroid hormones may find a practical application in the prevention or alleviation of symptoms of senile osteoporosis, in the acceleration of the healing of fractures or in the augmentation of the rate of somatic growth in certain hypogonadal individuals.

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CHRONIC MOUNTAIN SICKNESS

CARLOS MONGE

National Institute of Andean Biology, University of San Marcos, School of Medicine, Lima, Peru

The ecological milieu of the high plateaus of the Andes (2 to 5 km. altitude) must be emphasized as a fundamental factor when considering the biology of races acclimatized there since prehistoric times (Bingham). "Habitable" (Monge, 1928) places, in which life is naturally possible, must be differentiated from the higher "inhabited" ones, as for example the mines, in which conditions for living must be provided. Such a distinction has not yet been made. When studying acclimatization, much confusion would be avoided if the aim of the investigation were the physiological fitness of the autochthonous man in his ecological surroundings. The sufferances and tolerance of the living body to reach the highlands or to live at an extreme altitude should be considered as correlated subjects but ones of different significance. In no case should one use the words "acclimatized" or "acclimatization" when referring to an organism suffering from climatic anoxic injury during the adaptative stage (mountain sickness).

Congenital acclimatization to the Andean high plateaus (2 to 5 km. altitude), where live some twelve million individuals, has produced a climatophysiological variety of human being whose biological characteristics are somewhat different from those of the sea level man. Infertility of men and animals brought from the lowlands, as sometimes occurs (Monge), means the elimination of the unfit through a process of natural selection.

When a man goes to the highlands, he has first to adapt himself to a condition of permanent anoxia, calling forth his emergency adjustments and then building up a new set of biological devices to reach the balanced state of the altitude man homeostasis. Consequently, adaptation, itself, is a malady: mountain sickness, either inapparent, acute, subacute or chronic. When adaptation is over, after months or years, no one knows how long, acquired acclimatization supervenes. Then mountain sickness has been cured (Monge, 1928). Acclimatization can also be lost as a shift from the Andean man homeostasis, the patients developing subacute or chronic mountain sickness. If they are brought to a lower land or to sea level, chronic mountain sickness is also cured. In those facts one can find the strict foundation of a climatopathological variety of human disease (Monge, 1, a-h). Animals are subject to the same pathology and possibly plants, although no investigation has been conducted with them. Some men find it impossible to live even at an altitude of 1.5 km.: usually such men change their habitat and the observation ends. One could speculate about the condition of a man losing his acclimatization at sea level oxygen pressure.

To understand the physiopathological deviations during the adaptative period toward acclimatization, namely, mountain sickness, one must briefly consider the established characteristics of the autochthonous man. The emphasis will be laid upon the facts concerned with chronic mountain sickness which appears as

the basic cause of the problem. Since for some persons, apparently acclimatized, infertility may occur, the physiology of reproduction in high altitude will be treated.

Chronic mountain sickness, as has already been said, is a disease due to unfitness of the individual to become acclimatized at a high altitude or to a loss of acclimatization. Barcroft's work really dealt with sub-acute mountain sickness (2). I first used (1925) the name of Erythremia of high altitude when I had found an erythremic clinical syndrome similar to Vaquez' Erythremia or Osler's Polycythemia vera. This denotes a clinical entity and not a hematological pattern, as some have erroneously interpreted it. In using "erythremic symptoms," I refer to a clinical manifestation and not to a high count of red cells. In this review no attempt will be made to study acute mountain sickness[1]. Since I referred to the chronic condition as chronic anoxia, I called the disease "chronic mountain sickness" (1928) (1c, d, e). Later, this disease was studied by Monge, Encinas and associates, and Rondon (3). Talbot and Dill (4) found a case of it in Chile. Hurtado described some forms of the disease as related to fibrosis of the lungs (1930, 5a), and some to an erythremic syndrome (1942, 5b, c, d). Monge (1f) and Arrellano (6) have described neurological forms. Psychiatric forms have been described by Monge (1g).

In natives it may occur at any age and has an impressive familial character (Monge, Rondon). In individuals from the lowlands, the onset may come from two to three years after acquired acclimatization and generally appears in the fourth and fifth decades of life. Both sexes are equally predisposed. Certainly it is much more frequent in men of European descent. It may start insidiously or may come suddenly after violent physical exercise or after some respiratory ailment. Repeated attacks of acute mountain sickness predispose, as do probably pneumoconiosis, pneumonia and syphilis of the lungs.

I shall first describe the sub-acute forms, which, when cured, lead to altitude fitness. Then I shall consider the chronic stages of the disease.

SUB-ACUTE MOUNTAIN SICKNESS AND THE ANDEAN MAN. The anoxic injury acting on the organism causes an illness which, even in its mildest expression, leads to a lessened physical and mental capacity. To avoid confusion, I characterized (1925–1928) this as *acute*, *sub-acute* and *chronic* Soroche. This word in the *Quechua* language means mountain sickness.

Subacute mountain sickness is not always preceded by a preliminary attack of acute mountain sickness. In the most benign forms the patient goes on with his daily work, but he realizes that he is unable to maintain the life he was accustomed to at sea level. Even minor ailments provoke a run down condition never encountered at sea level. Diseases are poorly resisted and convalescence is pro-

[1] This is fully treated in: Haldane, J. S. and J. G. Priestley. Respiration, New Haven, Yale University Press, 1935. Barcroft, J. The respiratory function of the blood. Cambridge University Press, 1935. Loewy, A. Physiologie des Höhenklimas, Springer, Berlin, 1932. The pathology of high altitude climate. Oxford Medical Publications, 1937. Dill, D. B. Life, heat and altitude, Cambridge, Harvard University Press, 1938. Van Liere, E. J. Anoxia, its effects on the body. The University of Chicago Press, 1942.

longed. Toxic symptoms accompany pregnancy, delivery is dangerous, miscarriages are frequent. Infertility is common. In the more marked cases the symptoms appear insidiously and one or another predominates as, presumably, some particular organ suffers most damage from the prolonged oxygen want. General fatigue, cyanosis after exercise, diminished capacity for physical and mental work, fullness of the head, cephalalgia, dizziness, congestion of the mucous membranes, respiratory distress, poor appetite, slow digestion, constipation or diarrhea, loss of weight, insomnia, matutinal tiredness, relieved in the course of the day as in neurasthenia, are the most frequent complaints. Sometimes the patient may temporarily overcome his condition. After several weeks, months or years, the length of time is not yet established, there comes a marked improvement, sometimes abruptly. Very frequently the subject is unaware of his alleviation and finds himself fitted to carry on a normal life. He has become acclimatized. On the other hand, if the illness is more pronounced, fatigue comes sooner, cyanosis is permanent and nausea as well as vomiting may appear. Hoarseness and dyspnea are the rule. Torpor, cephalalgia, dizziness, vertigo, nervousness, insomnia, varied algesias and paresthesias appear. The patient, unfit for acclimatization, is suffering from chronic mountain sickness.

Circulation. In normal individuals, the second heart sound is duplicated very frequently. Rotta, by means of phonocardiography, has established the proper significance of this third sound as auricular. The heart rate tends to be markedly slowed (Monge, Cervelli). The pulse rate is 40–50 in 13 per cent; 52–60 in 41 per cent; 62–71 in 32 per cent; and 72–84 in 14 per cent (1927). This has been confirmed by Talbot and Dill (4), Aste (7), Cervelli (8), Torres (9), Arnaez (10) and Capdehourat and associates (11) (1937). The linear relation between work (700 *kgmts.*) and heart rate is only found in 30 per cent of the cases, with an even slower frequency than at sea level; 70 per cent show a kind of athletic response. After double the amount of work (1,400 *kgmts.*) the heart rate is slower than after a basic work of 700 *kgmts.* A paradoxic bradycardia occurs in 50 per cent of all cases after moderate work, then the pulse accelerates to reach the normal level (Monge, Encinas). Rondon found it to occur at an altitude of 5.8 km. Bradycardia sometimes comes suddenly. In 80 per cent of the cases tachycardia starts suddenly without any clinical disturbance. At any time the rate may return to bradycardia. Efficiency tests during the tachycardia do not interfere with the initial acceleration (Monge and associates 1h, 12). The electrocardiogram in two hundred observations shows, in general, normal values (Monge, Saenz, 13). There is a marked sinus arrhythmia, the mean difference between two systolic intervals may rise to 0.44. After moderate exercise (700 *kgmts.*), I have found exaggerated sinus arrhythmia, shift of the pacemaker, deformity of P, inverted P wave, shortness of PR, premature auricular and ventricular beats, voltage changes of T, reversal of T, and ST displacements. Some of these changes are similar to those found by Greene and Gilbert in acute experimental anoxia (1925). The subjects showed no signs of fatigue during the effort tests, notwithstanding the slow heart rates. Sometimes I have seen a regularly recurring arrythmia, prolonged (auriculoventricular) conduction, occasional nodal or high

bundle premature beats. In one case, the intraventricular conduction was altered. First degree block has been found. There is often right axis deviation. In one hundred cases Saenz found 40 per cent right axis deviation; Rotta found 37 per cent. The venous pressure is increased (Rotta, Capdehourat). There is a universal dilatation of blood vessels, rather pronounced in sub-acute mountain sickness. The arterial pressure in men is reported by Torres, on the basis of 100 observations, to be slightly lower than at sea level. Rotta's work (14) on circulation can be summarized as follows: *a.* Systolic output is approximately equal to that at sea level; *b.* The cardiac index, of greater importance (Cantoni), reaches 2.3 liters per minute, 12 per cent higher than the value at sea level.

The work of the heart in reference to surface area is increased. The volume (in men) and the weight (in dogs) of the heart is greater than at sea level. In sub-acute mountain sickness, tachycardia is the rule but the appearance of unexpected basal bradycardic rhythms (Monge, Cervelli) and paradoxic bradycardias after moderate exercise are common (Monge, Encinas). In such cases dyspnea is accentuated. At rest, the appearance of a third sound is easily noted. The second pulmonary sound is accentuated. Capillaries are dilated and lose their tortuosity. The arterial pressure is slightly increased with a lower diastolic value (Torres, 9). Pulsation of the head, as in aortic insufficiency, has been observed (Monge, 1928). The capacity for work is enormously reduced. Heart efficiency tests show retardation. Athletes, at high altitude, never maintain their sea level records for very long, as they are in the same situation as are untrained men at sea level. It must be emphasized that outside of the ecological milieu, even at the same altitude, the physiopathological deviations are more accentuated: therefore, in the future, it will be necessary to indicate clearly the places where research in chronic mountain sickness is being carried out.

Respiration. In the Andeans the chest is enlarged (Charvin, Barcroft (2), Hurtado (5e)) and vital capacity is 10 per cent greater than in Europeans (Barcroft). Linch (15) found in fifty Andeans that the respiratory rate is slightly increased above the sea level value. After moderate exercise, in 14 per cent, bradypneic rates started either following a short polypneic stage or at the beginning. Sometimes polypneic rates appeared without returning to normal values. In no case did the individual show a particular fatigue. The bradypneic stage is interpreted by Linch as an effort to increase the time of oxygenation. Arnaez (10) has pointed out, in addition to the well known periodic breath, apneic phases (25 per cent) at rest, deep sudden respiration (20 per cent), and staircase breathing as if the first breath were frustrated. This last finding is rather improved after moderate exercise. He suggests that the vagal hypertonia plays a more important rôle than at sea level.

These findings are the rule in sub-acute mountain sickness, and of course, are much more pronounced. Clinical observation shows that periodic deep breathing, accompanying periodic work, is the mechanism best suited to altitudes. Andeans can carry a load of a hundred pounds—they run, stop, take a few deep breaths, and run again. In subacute anoxia, it is possible to ameliorate the symptoms, reproducing the periodic hyperventiliation of the Andeans. Respira-

tory training is important. The respiratory adaptation to anoxemia has been studied by Forbes, Barcroft, Monge, Hurtado and Leon. Vital capacity is considerably increased (Hurtado and Leon). The most important work has been carried out by Hurtado and Rotta (16), from whom I quote a summarized report:

	ABSOLUTE VALUES		RELATIVE VALUES (TOTAL CAPACITY 100%)	
	3.7 km. altitude	Sea level	3.7 km.	Sea level
	liters	*liters*		
Resp. air	2.11	1.50	33.2	26.1
Mean capacity	3.58	2.89	56.4	50.3
Vital capacity	4.18	4.30	66.8	73.8
Total capacity	6.28	5.85		

According to them, the higher total capacity and the absolute and relative increase of residual air suggest a functional compensatory emphysema, as Hurtado pointed out (1928). This condition disappears at sea level, so it is not a truly acquired but an easily reversible characteristic. During subacute mountain sickness, the vital capacity is decreased, to rise progressively with amelioration. The respiratory form and rate acquire the modalities of Andeans after exercise, with the appearance of signs of dyspnea and asphyxia. Hyperventilation may lead to tetanus. One can observe the anarchy of the respiratory control: shallow respiration followed by deep breathing, periodic and Cheyne-Stokes breathing, sudden deep inspiration, apneic phases with sensations of impending asphyxia rather pronounced at night. The last may awake the patient and disturbs sleep (Arnaez, Linch).

Mori-Chavez (17a) has pointed out, in acclimatized guinea pigs, a hyperplasia of the capillary bed and diminished arteriolar structure which permit an increase of the O_2 diffusion surface; facts which must be related to the common observation (Orbigny) of a greater lung size. In the Andean, the chest x-ray has a characteristic appearance. The lung transparency is greater than at sea level, the hilus shadow is noticeably enlarged, and the pulmonary trunks are considerably emphasized, according to Garcia Rossell (18), and Saye and Monge. In sub-acute mountain sickness, this condition is more accentuated. In some cases pulsation of the hilus shadows appears on fluoroscopy. In chronic cases, the vessels' dilatation gives the aspect of pulmonary passive congestion. In some advanced cases, the x-ray films have the appearance of pneumoconiosis, which has led to erroneous diagnosis, as Jimenez has stated.

BASAL METABOLISM AND NERVOUS SYSTEM. While the basal metabolism of Andeans is normal according to Hurtado (5f), I have pointed out that, in moderate cases of subacute mountain sickness, there are often lower values which approach or reach normal metabolic rates with acclimatization. There is hyper-

tonia of the vegetative system, as proven by the oculo-cardiac reflex test (Monge, 1i; Pesce, 35a; Aste, 7), during which the Andeans do not feel any distress at all, despite a fall of 50 to 60 in pulse rate. In contrast, cases with moderate symptoms of sub-acute mountain sickness frequently collapse. In normal Andeans Pesce and I were able to give intravenously 3.5 mgm. of atropine without clinical symptoms. The increased vagal tonus seems to cause bradycardia after exercise. The parallel activity of the sympathetic adrenal system (Monge) may explain the tachycardias of sinus origin. The hypertonus of the vagal and sympathetic nerves must have some connection with the stability of the vaso-motor center and reflex nervous system. Gellhorn's findings on man in experimental anoxia can give an interpretation to the vertigo and the collapsing form of sub-acute mountain sickness. In spite of Hurtado's finding of a decreased gastric acidity, observation shows a hunger sensation after moderate exercise. Stomach ulcers are aggravated at high altitudes. Indigestion and diarrhea are common. Certainly some digestive disturbances may be due to the sensitization of the sympathetics by an increase of pH, as Van Liere (19) has suggested. Indeed a good deal more investigation of this type is needed (Van Liere).

Clinical observations show that changes in the psychological behavior at high altitudes are very frequent (Barcroft, Encinas), and several psychiatric conditions may result (Monge). McFarland (20) has carried out the most important research on the members of the International Expedition to the Andes. By means of psychological tests involving complex mental functions, he found reliable differences from performance at sea level. These manifestations are in harmony with the effects observed by the same author in flying or in pneumatic chambers.

On the basis of his experiments, Hahn (21) (of the National Institute of Andean Biology) states that it is a mistake to generalize, since some subjects are not affected by altitude. All psychological activities are slowed and it is difficult to maintain attention (Ziehen's method). Even a very complicated task, involving eye and hand co-ordination (mirror drawing of Giese), could be carried on in the mildest forms of sub-acute mountain sickness but more time was required. Mental training and automatization seem not to be affected. He emphasizes that in color naming (McFarland's method), if the verbal expression is substituted with a wordless procedure (Hahn's), the rate of errors is not affected by altitude. Regarding Andeans, there are no marked differences in visual reaction time, but lower motor speed values (tapping of Whipple) are found. As regards attention, he found clearly lower values than at sea level (Toulouse, Pieron's method—unpublished work).

Blood acid-base balance. Reported changes of acid-base equilibrium produced by hyperventilation, based on hydrogen ion concentration observations, are not in agreement because researches have been carried out with subjects whose acclimatization had not been entirely determined. Some of them evidently suffered with sub-acute mountain sickness. Monge pointed out (1928) that adapted people have a higher serum pH value than acclimatized people. Dill, Christian-

sen and Edwards (22) found in adapted men a tendency for a slight increase in pH, progressive with altitude to about 4.6 km. Above this level the pH diminished but was higher than that of acclimatized residents.

Aste reported the same facts in chronic mountain sickness at 3.2 km. altitude. In general, the blood pH value is found within normal limits with a marked tendency to be on the alkaline side. Aste, as will be seen later, found a greater difference of pH between arterial and venous blood in Andeans than the normal value at sea level in his studies on chronic mountain sickness. However, Dill, Talbot and Consolazio (23) found normal values at a higher altitude. Dill, Talbot and Consolazio showed that in habitable places, both CO_2 and alkaline reserve diminished, but never in sub-acute mountain sickness did the fall reach the lowest values of the residents; according to them, months will be required to reach these values. With very crude methods, V. Villa-Garcia (1935) and I (1j) arrived at the same conclusion, measuring the increase during adaptation. Since 1928, I have emphasized the fact that, in spite of a minimal p CO_2 and CO_2 content, the blood of Andeans has a higher buffer capacity than that of individuals with sub-acute mountain sickness. At least this capacity is equal to that of the sea level man who has a much greater p CO_2 and CO_2 content. Probably, there must be a readjustment of buffers, the nature of it being unknown; thus the buffer system must be compensated. The increased hemoglobin might account for some of the difference according to Dill, Talbot and Consolazio (23). They state that there is a deficit of 2 milli-equivalents in the balance of electrolytes, at 5.3 km. altitude. However, the problem is not yet solved. Certainly the organism as a whole must find some new regulatory mechanism to stabilize the internal respiration as a balanced mirror of the external environment. The different steps described by Y. Henderson, as a consequence of decreased oxygen pressure, have been outlined in sub-acute mountain sickness by Monge (1928) who established the reciprocal relation between the blood alkaline tide due to diminshed CO_2 and the elimination of the bases through the urine as measured by pH ammonia, and titratable acidity. A reversible process is found on returning to sea level (Monge, 1b). I have observed in Andeans (1928–1935) that the shift of pH after moderate exercise is equal to or less than that attained at sea level. In sub-acute mountain sickness it is considerably greater and diminishes with acclimatization, as has been proved by standard exercise performances (Monge, 37).

Andeans show a high capacity for work. "Every few minutes one would appear (Cerro de Pasco, 14,700 ft. altitude) carrying a weight on his back of a hundred pounds brought up from 250 feet lower down" (Barcroft). The reviewer insists upon these facts because the literature of this subject is full of erroneous statements since the time of Jourdanet and Barcroft. The Andean is constructed like an athlete. This means that sub-acute mountain sickness is a disease of temporary or permanent disability, a fatigue disease. As in athletics, acclimatization means fitness for continuous anoxia. Mountain sickness suddenly changes the endurance of a trained individual into an unfit, unable to reach a high pulse rate as at sea level, or to hyperventilate adequately to permit suitable

work of the heart (Christensen, 36). Certainly the transport of CO_2, the enzymatic activity of the internal milieu in fixing O_2, and a greater cardiac output, must play a most important rôle in the altitude homeostasis, as Dill suggested (24).

Blood. Barcroft (2), Heraud (1k), Hurtado (5a–g), Hurtado and Guzman Barron (5h), Talbot (25), Capdehourat (11) and Aste (unpublished work) have confirmed the high altitude polycythemia found by Viault and have studied the blood morphology and hemoglobin, which do not differ from the situation at sea level (Hall, 26). I have summarized the most recent observations in table 1.

It can be seen that the red cell count, hemoglobin and hematocrit are higher than at sea level. Hurtado has calculated the several blood variables. He pictures the erythrocyte as a cell of greater size with less hemoglobin and oxygen saturation to facilitate the gaseous interchange. In general, the results need to be revised in relation to ecological surroundings. In sub-acute mountain sickness, Monge has found a higher count of erythrocytes than the normal value at

TABLE 1

CONDITION	AUTHOR	ALTITUDE	R.B.C.	HEMOGLOBIN	HEMATOCRIT R.B.C.	M.C.V.	M.C.Hb	M.C.Hb CONCENT.
		meters	*millions*	*grams per cent*	*per cent*	*cu. microns*	*γγ*	*per cent*
Acclimatized	Hurtado	4,500	6.66	15.93	71.1	96.20	24.4	24.9
	Aste	3,200	5.85	17.69	47.9	82.60	30.5	36.9
Subacute mountain sickness	Talbott	3,660	5.54	17.23	49.6	89.50	31.1	34.7
	Talbott	4,710	5.84	17.90	53.4	91.40	30.6	30.6
Chronic mountain sickness	Aste	3,200	6.54	20.92	56.2	86.46	32.2	37.2
	Hurtado	4–5,000	8.26	24.70	77.5	94.1	30.3	32.2

the same altitude (3.7 km.). This may be deduced also from Talbot's data at 2.8 km. in the Andes. Talbot's observations show a progressive increase of the count up to about 5 km. altitude, but it never attained the level of residents. Higher up, at 6.1 km., the count was lower. In general, when the adaptative stage reaches an upper limit and acclimatization does not come, the number is enormously increased. The hemoglobin follows a parallel curve. Talbot suggests that to reach a maximum of hemoglobin months or years may be required. Hurtado found in Andeans a blood viscosity of 8.6 (± 0.14); a coagulation time of 6.5 (± 0.12); a serum bilirubin of 0.7 mgm. per cent. Urteaga (unpublished work) has found in 6 cases a bilirubinemia average of 13.7 per cent; direct Van der Bergh 4 per cent; indirect 9.7 per cent. He considers this increased threshold of bilirubinemia as normal since the excretion bilirubin test gives normal curves, if referred to this higher threshold. He suggests it might be a device to save the pyrrholic nuclei needed in hematopoiesis (Urteago, 27). Palti (28), at 3.2 km. in one hundred native Andeans, has found a calcium value of 11.62 (± 0.05) mgm., slightly above that of 11.06 (± 0.06) at sea level. The

active Ca was higher at the high altitude. In sub-acute mountain sickness, hypercalcemia, returning gradually to normal, was noted in severe cases. Salas (29), in fifty natives at 3.2 km., has found higher blood protein values than at sea level. In sub-acute mountain sickness the proteinemia was still higher. Perhaps his observations did not last long enough to encounter a reverse change. San Martin (30) has confirmed in seven individuals the slight blood sugar increase found first by Forbes (31) in sub-acute mountain sickness, and in addition has noted higher values of blood corpuscle sugar compared with sea level values. He found 0.71 mgm. per cent, instead of 0.59 mgm.; in hematocrit value, 0.97 mgm. instead of 0.46 per cent. Probably with acclimatization blood sugar may come back to lower levels, as was found by Forbes. There are not enough data to make definite conclusions. I have found the white cell count normal in Andeans but slightly increased in sub-acute mountain sickness. Most impressive are the increased number of reticulocytes and the presence of an enormous number of histiocytes in mild forms of altitude disease. Even in normal men they have been present (Hurtado, Barcroft, Monge, Talbot).

Barcroft's work regarding arterial blood O_2 saturation established that the Andeans had a lower value (80–82) than newcomers (90–92). Hurtado confirmed this, as did different members of the Chilean International Expedition. The most extensive work has been done by Hurtado, who has established particular levels of arterial blood O_2 for different altitudes. Aste, at 3.2 km., has found saturations more like those at sea level (93.5). Dill, Christensen and Edwards (22) have confirmed Barcroft's view that diffusion alone accounts for the transfer of gases in the alveolar epithelium. Keys, Hall and Guzman Barron (32), working at several progressive altitudes, have stated that the "physiological" deviation curves tend to shift to the left of sea level values until about 4.5 km. altitude and to the right at higher stations. "This may or may not be related with the fact that somewhere around this altitude there is not a relationship between O_2 arterial saturation and the well-being of the subjects." On the contrary, the natives have the lower saturation. So, in order to explain the inability of the body to fix oxygen in mountain sickness, Guzman Barron, Dill, Edwards and Hurtado (33) suggested a disturbance of the oxidation-reduction system. Edwards (34) argued that the problem is one of utilization rather than of transportation and suggested that myoglobin plays an important rôle. In fact, Hurtado, Rotta, Merino and Pons (35) proved this to be true as regards the myoglobin. Since Campbell's research, particular attention has been paid to the oxygen capillary pressure. In discussing acclimatization, I reported higher venous O_2 saturation and pressure in cases of sub-acute mountain sickness as compared to Andeans, as if the tissues had not taken oxygen from the blood (1928) (1a). Aste (unpublished work), comparing the arterial and venous O_2 saturation of normal and chronic mountain sickness individuals, has found facts in agreement with my previous investigations. Thus he points out in acclimatized men an arterial-venous O_2 difference of 60.5, as compared with only 39.8 in patients. In spite of the fact that there are other factors which can influence the uptake of O_2 from the blood, it is interesting to point out that a characteristic of

mountain sickness is that the O_2 flows by without being taken up by the tissues. The same holds true for patients suffering with sub-acute mountain sickness who, on being brought to sea level during the recuperative period, show a diminished O_2 intake. These facts were so impressive that the reviewer could suggest (1928) that "altitude changes the capacity of the tissues to fix O_2. We can suggest that there are unknown substances which bring about acclimatization" (1a). This opinion still is in harmony with the modern concept of biochemistry (38).

Chronic mountain sickness. The clinical picture resembles fully advanced cases of Polycythemia vera. The patient, at rest, has either an erythrose color, which turns blue on the least effort; or a dark cyanotic color, more conspicuous in the face and hands. There is a generalized dilatation of the blood vessels. The sclerae are intensely colored by distended capillaries, the eyes being hidden behind edematous and bluish eyelids. The nasal and oral mucosae are wine red. The skin of the body is dry, while the forehead and hands are usually covered with sweat. The hands show clubbing of the fingers. The nails appear to be inserted like watch crystals. Epistaxis is frequent, hoarseness and aphonia are often noted. The thorax is enlarged—of the emphysematous type. The patient feels extremely weak, has a tendency to sleep and is frequently found in a state of drowsiness. Sometimes he falls into coma for two or three hours. Spells of dizziness and faintness occur. On occasions there are crises of vomiting. Constipation alternates with diarrhea. Dyspnea is permanent, bronchitis is frequent. There are recurring congestive processes of the lungs with hemoptysis and low fever, which disappear when the patient goes down to sea level. Cardiac disorders are not found for a long time, but right heart insufficiency supervenes with the progress of the disease. Cardiac pains of anginal distribution appear in some cases. In one case, simultaneous with angor, the vision grew cloudy and the patient became unconscious and fell. Hyperthermia very rarely occurs. Temporary blindness (Dammert) and temporary deafness are not infrequent. The liver is moderately enlarged. Enlargement of the spleen has been found in 12 per cent of the cases.

Algesias and paresthesias are a common complaint. Excruciating pains in the extremities and pain in the lumbar region or in the joints, particularly in the tendon attachments at joint cavities, are present. The pain sometimes immobilizes the patient during several days or weeks, only to disappear spontaneously or when descending to a lower altitude. Bleeding improves this condition. Some of these symptoms which I described (1928) are similar to those found by Armstrong in aviators. There may be violent cephalalgia, subsiding after lumbar puncture. Paresthesias are varied in type and localization: unpleasant sensations of heat in the face or of hot water being poured on the back. One patient had the sensation of the loss of one hand. Formication in the feet and the sensation of being pricked by pins are frequent.

In cases of severe involvement it is possible to find marked disturbance in the behavior and memory of the patients—the entire psychic personality appearing altered. Nervous exhaustion is very common. The patients complain of sexual frigidity.

Marked polycythemia and augmentation of the blood volume is always found (Hurtado, 5). The red cells, as a rule, are between seven and twelve million (Monge). There is slight leucocytosis and eosinophilia. Some cases show alteration in the clotting with bleeding of the gums, purpura with central nervous symptoms, gastrorhagias, metrorhagias, hemoptysis, etc. Renal complications may occur as well as albuminuria and hematuria.

In an important work, Hurtado (5d) describes the blood findings and discusses the etiological mechanism of chronic mountain sickness. He has pointed out an absolute polycythemia with hypervolemia. Hurtado, Talbot and Aste found, at an altitude of 4 to 5 km., hyperbilirubinemia (from 2.2 to 8.3); hyperviscosity (1.6 to 20.8); arterial O_2 saturation from 75.9 to 82.9; blood volume from 157.1 to 211.9; CO_2 content from 29 to 35.7. When the patient stays at sea level, the cell volume diminishes and the plasma increases up to the normal equilibrium. Hurtado does not believe that there is any similarity between the polycythemia of chronic mountain sickness caused by oxygen unsaturation and that of polycythemia vera due to a disordered activity of the entire hematopoietic system. Since he did not find a great increase in residual air which characterizes pulmonary emphysema, he ruled out this too as a cause of polycythemia. He states that the cause for chronic mountain sickness might be found in sclerotic changes of the lungs.

Aste (unpublished work) has made an important study of the arterial and venous blood changes in chronic mountain sickness on the heights and during sea level cure. Comparing arterial and venous blood of acclimatized persons and of cases of chronic mountain sickness at 3.2 km. altitude, he found (table 2) that all the hematological constants of chronic mountain sickness show higher values in venous blood. The arterial O_2 saturation is diminished but venous O_2 saturation is high, so that tissue O_2 intake is reduced. He found, as has been said, a rather pronounced difference of the pH value between arterial and venous blood (from 0.05 to 0.09). The alkaline reserve is higher—42.5 in chronic mountain sickness, compared to 40.1 in Andeans of the same altitude. The hematological study of patients during sea level cure shows: a diminution of arterial and venous blood cells; an initial rise and then a fall in cell volume and oxygen capacity. The arterial venous O_2 difference is smaller than that in normal Andeans. With amelioration, venous O_2 saturation goes down, as if the tissues had reacquired their capacity to take O_2 from the blood. This is an impressive fact, not due to heart insufficiency and always parallel to the amelioration of the disease. After an initial phase of increased arterial pH, both arterial and venous pH stabilize themselves at the approximate figures for sea level.

Urteaga (27) has arrived at the same conclusion as regards patients brought to sea level. He emphasizes the fact that with the sea level cure, the macrocytic hyperchromic cells change to smaller cells with high hemoglobin. The hyperbilirubinemia which he found goes down step by step. In each case injected bilirubin gave a normal curve compared to the higher threshold considered normal at high altitudes. All authors agree to the presence of a great number of histiocytes and reticulocytes. The white count shows a leucocytosis and often

an eosinophilia. Certainly there is established at high altitude a new equilibrium between the increased hematopoiesis and the increased destruction of red cells.

The characteristic feature that gives this disease its personality is the fact that all troubles and ills disappear when the patient is brought to a lower altitude or, of course, to sea level. This undoubtedly is due to a single cause: anoxemia. Usually after a stay at sea level, the patient can return to the altitude and live there for some time without great discomfort. As time goes on, however, the sea level cure fades and asphyxial disorders conducive to immediate death in coma and acidosis may occur as soon as the patient reaches high altitude. Most of the patients become acclimatized temporarily at a lower altitude, but at times they have to go down to sea level.

I have described several varieties of chronic mountain sickness, according to the predominance of oxygen want in certain tissues or organs. I shall enumerate them briefly. In the congested cerebral form (1f) appear (1936) spasms of painful headache, fullness of the head, photophobia, capillary ocular injection, blur-

TABLE 2

BLOOD	CONDITION	R.B.C.	HEMOGLOBIN	HEMATOCRIT R.B.C.	M.C.V.	M.C.Hb	M.C.Hb CONCENT.
		millions	*grams per cent*	*per cent*	*cu. microns*	γγ	*per cent*
Venous	Acclimatized	5.85	17.69	47.9	82.6	30.5	36.9
	Unacclimatized (chronic moun. sickness)	6.54	20.92	56.2	86.4	32.2	37.2
Arterial	Acclimatized	6.14	17.50	47.3	77.5	28.7	36.9
	Unacclimatized (chronic moun. sickness)	6.21	20.21	57.4	92.5	32.7	35.3

ring of vision or deafness, scotoma, lacrimation, vertigo, dizziness, general sweating and vomiting, intense cyanosis, bradycardia, drowsiness and unconsciousness. The spinal fluid pressure is enormously increased. Arellano (6) has established that repeated lumbar punctures relieve the patient. Frequently spontaneous epistaxis ends the crisis. Neurological forms, with monoplegia or hemiplegia, sometimes appear. In one case, after several months of moderate erythremic symptoms, the patient suddenly developed a modest degree of palsy of the right arm and aphasia lasting several minutes. Every morning for eight days the trouble returned. On coming down to sea level the symptoms disappeared. Two months later the patient returned to high altitude but after a stay of three weeks the troubles reappeared and he was obliged to give up his work. Now he is entirely normal after two years at sea level. Collapsing forms are frequent and usually are associated with a syndrome of hyperventilation and tetany. In one case of moderate erythremia at 4.6 km., two attacks of tetany occurred at an interval of several years, provoked by a light bronchial inflamma-

tion. The condition was so severe that the patient, in status tetania, was transferred to a lower altitude (2.8 km.) and the symptoms disappeared during the trip.

Cardiac, digestive and pulmonary emphysematous forms are frequent. I have observed one case of erythremia with crises of intense cephalalgia (once relieved by lumbar puncture) accompanied by albuminuria, all of which disappeared when the patient came to sea level. The symptoms and the albuminuria recurred again at the high altitude. At sea level during seven years the patient remained normal. Recently he returned to high altitude and developed a syndrome of high arterial pressure, without renal insufficiency, which was cured on returning to sea level.

Mental forms are common (Monge, lg). In a recent case a change of the personality was surprising. The patient found himself unable to work; he saw everything wrong; he was afraid to meet his employees. Sometimes he got up at night and pretended to work. He realized that there was nothing to do but he went just the same. Finally he conceived the idea of committing suicide. He was immediately relieved on being brought to sea level. The mental disturbances came back when he returned to the high altitude. At sea level he now enjoys a permanent cure.

When one considers the clinical syndromes which lead to polycythemia, a clear division into two groups may be established. To the first group belong the lung and cardio-arterial processes which act mechanically on the permeability of the respiratory epithelium. Its symptomatology, besides polycythemia and changes of the acid-base equilibrium, is distinctly pulmonary. In the second group there is polycythemia and a definite clinical syndrome—the so-called erythremic syndrome (Vaquez, Osler, Monge) with its ubiquitous pathology never found in the first group. These two diseases of equal nosographic symptomatology, polycythemia vera and chronic mountain sickness, have different pathogenesis: the cause of the second is clear—anoxemia. No one knows the cause of polycythemia vera. After my first paper (1935), I was inclined to accept the identity of both because there is a gradient of frequency of cases from the high altitude "inhabited" places to the lower "habitable" places and finally to sea level. Thus I do not see any reason to discard the possibility of the loss of acclimatization at sea level. It would remain to be demonstrated that oxygen therapy relieves selected cases of Vaquez disease at sea level. Unfortunately the therapy done in compressed chambers is not conclusive. Several months, at least, are needed to have a steady amelioration at sea level. Hypertension and enlarged spleen may be found in chronic mountain sickness.

As a hypothesis of pathogenesis I suggested (1928) that impermeability of the lungs is due to a low diffusion coefficient of O_2, as can be deduced from Harrop's work (39). However no proof has been given. Hurtado (5d) in his last paper stresses the importance of lung fibro-sclerotic changes due to anoxemia, increased arterial pulmonary pressure and high viscosity. He thinks that morphology has been unduly neglected. Structural changes of the lung alone, as in

pulmonary sclerosis, would not explain the universal cellular pathology which occurs in chronic mountain sickness. So it may be a primary chemical dysfunction. If the hypothesis of Guzman Barron, Dill, Edwards and Hurtado concerning the alteration of the oxidation-reduction systems due to low O_2 pressure, which is supported by the findings of Monge (1928) and Aste (1940) regarding O_2 not taken by the tissues, is accepted it must hold for lung tissue metabolism. Certainly the lining of the respiratory cells which regulates O_2 passage from the alveolar air to the blood must be included in the same enzymatic dysfunction. Besides, this hypothesis must be extended to the mechanism which regulates the transport and elimination of CO_2, for carbonic anhydrase according to Meldrum and Roughton plays a decisive rôle in the output of CO_2 from bicarbonate in the lungs. Dysfunction would mean disturbance of CO_2 elimination, which always occurs as soon as mountain sickness begins. There is not any fact suggesting a deficiency of mineral traces. Normal enzymatic equilibrium explains the whole process of the acclimatized organism integrated by hormonal and nervous devices. Its failure would mean chronic mountain sickness, either ubiquitous or elective as it happens to be, in the case of lesions of the germinal cells.

The lasting treatment is the cure at sea level. I have tried x-ray therapy to shorten the time of recuperation.

Inapparent chronic mountain sickness and the physiology of reproduction at the high plateaus. It is a very well known fact in South America that high altitude exercises a deleterious influence on fertility. Clinical observations prove it in man and in animals (Monge, 40f). I have described cases of fertile couples at sea level who were infertile at high altitude. In one case the infertility of the male was demonstrated. Carvallo (personal communication) found two cases of sea level men coming from high altitude, in which no spermatozoa could be found. It is a common fact that adapted pregnant women often come down to the coast to be delivered because miscarriages and sometimes sudden death of the new-born happen at the heights. In animal breeding I have pointed out (1940) some interesting facts. Eggs from sea level do not hatch at high altitude (Humboldt, Agazzoti). A couple of dogs brought to Lassa (Thibet) never reproduced according to Cutting (41). Sterility in rabbits, horses, cattle and cats has also been found.

It is interesting to recall Father Calancha's observation (1639) that the Spanish conquerors at 14,000 feet (Potosi, Bolivia) did not have offspring until fifty-eight years after the city was founded. In 1535, the capital of Peru was transferred from Jauja (11,500 ft.) to Lima (sea level) because the horses, fowl and pigs did not reproduce, as is stated in the act of foundation of Lima. Father Cobo (1605) established Mendelian anticipations in saying: "The Indians are stronger and multiply enormously while the majority of the children of Spanish parents born in such climates do not live. . . the climate's influence is visible in the half-breed and quarter-breed; . . . the more Indian blood, the better they grow (40a)." On the other hand, the birth rate of the Andeans is equal to that

of the sea level people. I quote from the last census of Peru, the following figures through the courtesy of Dr. Arca Parro:

	ALTITUDE		
	0–2 km.	2–3 km.	3–4.4 kgm.
Population	2,251,718	1,596,004	1,742,229
Birth rate %	28	26	26

Thus the physiology of reproduction in the high plateaus is normal in regard to the birth rate of the Andeans.

Research in the physiology of reproduction related to subacute, chronic, and particularly inapparent chronic mountain sickness has been conducted by Monge and associates (40b). Monge and Mori-Chavez (40e), on examining cats sent to 4.4 km. altitude, found that the testes were devoid of the germinal epithelium, Sertoli cells replacing spermatogonia. Even basal spermatogonia had disappeared. The Leydig cells showed a noticeable increase. In some rabbits they found that the majority of the seminiferous tubules had only the basal line of spermatogonia and Sertoli cells. In others, there was a second row of spermatocytes but neither spermatocides nor spermatozoa. In some rabbits the process of recovering was visible.

The research in sheep planned by Monge (40c) and conducted by San Martin (42) is very promising. Sheep acclimatized at high altitude since the seventeenth century have 100 per cent fertility. The fertility comes down to 30 per cent when pure-blooded imported rams, from sea level, are used for cross breeding. With progressive adaptation, fertility can be raised to 60 per cent to 70 per cent. But in order to attain this result 4 to 7 rams per hundred are used instead of the one or two at sea level. So there is in general a very high percentage (40–50 per cent) of infertility which, according to current opinion, is due to the ewes being infertile ("machorras"). This percentage, in my opinion, could be the statistical result of ewes mated with rams of unproven fertility. Actually when the rams are brought to a lower altitude they recover their fertility. From the research now in progress, two facts deserve special mention. In a group of 67 supposedly selected acclimatized rams, the variation of semen pH was 5.6 to 8.5, which indicates that the pH regulatory system fails at high altitude. The motility was diminished and the sperm was not suited for insemination. Only in 23 was the pH within normal range: 6.2 to 6.8. Recently, with the aid of San Martin, a study has been made of 2 rams selected as sires in Chile (sea level altitude) and brought to Huancayo (3.2 km. altitude). Their food was selected; both suffered from mountain sickness during the first week. Then they were apparently normal. One, after eight months, does not have spermatozoa but has an exaggerated sexual desire. The other, after an initial stage of reduced spermatogenesis (twelve million germinal cells instead of a thousand million), high pH (8.5), and absence of motility, has recovered. However, he has very low sexual desire. Easly, on the other hand (unpublished

work), tested a group of rams recently brought to an altitude of 4 km. and found 40 per cent of them fertile.

By selecting normal semen and artificial insemination, it was possible to get 86.4 per cent pregnancy in the supposedly infertile ewes ("machorras"). (San Martin). Thus was proved the infertility of the males and the fertility of the females ("machorras") (1941). The emphasis given to this point is explainable because it concerns eleven million sheep in Peru alone.[2] In a field experiment at the altitude of 4 km. with only one selected ram, seven hundred ewes were mated with a resulting pregnancy of 68.8 per cent. The control group, seven hundred ewes mated with 49 rams as usual in the high plateaus and under natural conditions, gave only 60 per cent pregnancy. So animal races can be created through scientific discrimination.

Here I enter on particularly interesting ground: race pathology, as a function of inapparent clinical disturbances. Chronic mountain sickness cannot be judged only by a general appearance or by an outstanding clinical symptomatology. Its influence may be extended electively to the germinal cells. In high altitudes men, both physically and mentally, might seem to be at their best but at times they are sterile. In animals the same thing occurs. Even though the sexual desire is manifest, the observer may be misled. It is not an exaggeration to stress the gravity of these findings in regard to the problem of population and the possibilities of human and animal races not suited for acclimatization. One is obliged to accept the fact that the autochthonous Andean man belongs to a climatophysiological variety of human race which has adequate power of reproduction in an atmosphere of permanent anoxia. This may help to explain: the attachment which that man has for his altitude environment, the ailments suffered by him in the traumatic climate of the lowlands (lowland sickness) and the urge to return home that has made of him a nomad, and finally the old Inca mandate that permitted mass population displacements only to similar climates—a factor still active in the annual work migrations. Certainly, the Incas knew more about the influence of the high plateaus upon the individuals and the behavior of the autochthonous societies of America than do men of today (Monge). Acclimatization has a biological determinism on the individual, the race, the societies and the history of South America (Monge, 40d). The unfit are eliminated through a process of natural selection (chronic mountain sickness), either visible or inapparent.

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[2] I am very much indebted to Prof. Fred McKenzie of the Utah State Agriculture College, Logan, Utah, who kindly came to Peru to aid in this research.

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